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AN EXPERIMENTAL AND CLINICAL STUDY OF THE VALUE OF PHENOLTETRACHLORPHTHALEIN AS A TEST FOR HEPATIC FUNCTION.

By L. G. Rowntree, M. D., S. H. Hurwitz, M. D., and A. L. Bloomfield, M. D., Baltimore.

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In this investigation an effort has been made to determine whether the quantity of phenoltetrachlorphthalein excreted by the liver following its intravenous administration affords an index of the functional capacity of the liver. The specificity displayed by the liver in the excretion of this dye, which is analogous in every way to that exhibited by the kidney towards phenolsulphonephthalein, strongly suggests possibilities in this connection. Quantitative studies of the phthalein output in health and in liver diseases (clinical and experimental) have therefore been undertaken.

The Function of the Liver in Health.

The liver plays an important rôle in the general nutrition of the body. No anatomical or functional differentiation of liver cells exists, all being identical as far as can be determined. Three functions of liver cells are definitely established: (1) The glycogenic function, relating to carbohydrate metabolism. This consists of (a) the conversion through enzymatic activity of monosaccharides (dextrose, levulose and galactose) brought to the liver cells by the blood, into glycogen, a polysaccharide closely related to starch; (b) the temporary storage of glycogen as such, until (c) the reconversion of glycogen by liver enzymes into dextrose as need arises for sugar throughout the body. (2) The formation of urea in relation to nitrogenous metabolism through the activity of the liver cells. This consists in the conversion of certain nitrogenous bodies (NH_3, amino acids, etc.) into urea, which in turn is carried to the kidneys, where it is excreted. (3) The formation of bile, which is in part an excretion carrying with it waste material and in part a secretion concerned in digestion, playing an important rôle, particularly in the absorption of fats.

Other functions are frequently ascribed to the liver. Their connection with it, however, is not so well established and they have not played so important a rôle in the studies of liver physiology. In this group must be considered the formation of fibrinogen and of antithrombin. Undoubtedly still other important functions exist of which at present little or nothing is known.

The Function of the Liver in Disease.

With the occurrence of disease in the liver, functional changes undoubtedly appear. Their character varies greatly with the nature of the underlying pathological processes at work. They can be associated with, or totally independent of, morphological changes, macroscopically or microscopically demonstrable. In most of the outspoken diseases of the liver, however, recognizable objective anatomical alterations occur,

such as enlargement or contraction, changes in consistency, etc. They may or may not be associated with evidence of portal obstruction, ascites, the development of enlarged collateral circulatory channels or of biliary obstruction—jaundice, bile in the urine, acholic stools, etc.

Through routine clinical histories and examinations, with a study of the urine and fæces, the presence or absence of liver disease can be readily determined in most cases. Information concerning the severity of the disease and the extent of the involvement of liver function is, however, not so readily obtained. In certain liver affections symptoms either of an obstructive or toxic nature are ascribed to the liver changes. There is not, however, a well-defined symptom complex which can be accepted as the picture of hepatic insufficiency, such as exists, for instance, in relation to diseases of other viscera—the kidney, heart, suprarenals and thyroid. In other words, the clinical picture and anatomical changes, even when outspoken, do not furnish an accurate conception of the functional condition of the liver, nor do they furnish reliable criteria concerning the outcome of these functional changes.

In certain other diseases, concerning the etiology and physiological pathology of which no accurate knowledge exists, the liver is thought to be implicated. Information about hepatic function in these conditions is most desirable and necessary.

The unsatisfactory character of the information obtained through ordinary routine clinical studies is apparent to every thoughtful clinician. The desirability of broadening and deepening our acquaintance with the functional conditions present in disease is indicated by the existence of a large number of tests devised and introduced for this purpose.

The Ideal Functional Test

A perfect conception of the status of liver function in disease presupposes an accurate knowledge of all the physiological functions of the liver in health and in disease. Do dissociated injuries to one function or set of functions exist without interference with other functions? If so, reliable quantitative tests for each set of activities is demanded. Our knowledge of liver physiology, however, is inadequate and much information, fundamental in nature, is needed before such tests can be devised.

It is worth while to consider what would constitute an ideal single test of total liver function. Is such a test possible? What could be justly demanded of it?

1. The test should indicate within narrow limits a constant amount of work performed by all normal livers under normal conditions.

2. It should indicate constant variations in function where constant abnormal conditions of either an experimental or clinical nature exist.

3. It should indicate functional alterations independent of the histological appearance.

4. It should afford an indication of the absolute work accomplished as well as the relation of this to the normal standard under all conditions; that is, it should indicate correctly the degree of functional injury, thus carrying prognostic significance.

5. Where less than the minimal amount of liver capable of carrying on function is left free from disease or injury, corresponding lowering of function should be indicated.

6. Where all liver cells are diffusely involved, lowered function should be indicated, but where certain cells are injured while others take on, through compensatory activity, additional function, the total functional capacity alone should be indicated.

7. It should be applicable with as simple technic as possible, so as to be available for general use in all forms of liver injury.

8. It should be applicable without injury of any kind (local or general) to the patient and without placing the liver under any additional strain.

9. The method itself should be mathematically accurate.

10. Its results should be easy of interpretation.

11. Its results should not be subject to influence from involvement of any other organs or systems, except in so far as the liver function is secondarily affected; that is, the test should be specific for liver changes.

The Tests of Liver Function.

Numerous tests have been employed in the effort to determine the functional capacity of the liver in disease. They are mostly based upon the physiological functions of the liver and attempt quantitatively or qualitatively to determine its capacity along such lines.

The Carbohydrate Tests.

The discovery of the glycogenic function of the liver in 1857 by Claude Bernard immediately stimulated extensive work in carbohydrate metabolism by physiologists, pathologists and clinicians. During the course of a rather heated controversy which waged in the German and French literature between 1875 and 1900 concerning the relationship of the liver to glycosuria after the administration of large amounts of carbohydrate, the idea of utilizing the sugars for testing hepatic function arose. The French school led by Roger,[3] Achard and Castaigne, Baylac[3] and Bierens de Haen[3] championed the sugars as tests of liver function, while the German school under the leadership of Quincke,[4] Frerichs, von Noorden,[5] Kraus and Ludwig,[6] Bloch,[7] and Müller, were unable to demonstrate any marked or constant reduction in sugar tolerance in cases of liver disease.

In a series of papers in 1898-1900 Strauss[8,9] established the view that the discrepancies in the results of these various workers could be explained by differences in the particular carbohydrate employed, together with differences in the amounts of sugar administered. He demonstrated that 100 gm. of dextrose in 500 cc. water given on an empty stomach gave rise to glycosuria in but two out of 38 cases of liver disease, whereas a considerable proportion of all

hepatopathies showed the presence of sugar in the urine following the administration of the same amount of cane sugar. He ascribed the difference to the presence of levulose in the cane sugar. He concluded that both cane sugar and dextrose were inapplicable, since there existed a mechanism other than that in the liver capable of metabolising them. He followed his criticism of this work by his levulose test, based on the work of Sachs,[10] which showed a constant decreased tolerance for levulose in liverless frogs.

STRAUSS' LEVULOSE TEST.

One hundred grams of levulose are administered on an empty stomach, and the urine voided during the following four hours tested by Trommer's and Seliwanoff's tests, by fermentation and polarization. The normal individual should tolerate 100 gm. levulose without glycosuria. The test came rapidly into wide use, the results differing with various workers. A contrast between the findings in health and disease, together with the attitude of various workers concerning the value of the test, is seen in the following table.

THE LEVULOSE TEST.

Author.	Normal livers.		Abn'rmal livers.		Remarks.
	Cases.	Positive.	Cases.	Positive.	
Strauss[8 9]	58	6	25	23	Test is of value.
Ferranini[11]	..	..	16	15	Test preferable to glucose test, which showed only 10 positive.
Landsberg[12]	7	4	21	9	He thinks that normally tolerance varies so much that test is of no importance.
Chajes[13]	21	2	..	..	He thinks that positive findings are rare in normals.
v. Halasz[14]	20	1	23	8	The 8 positive findings were in cirrhosis; considers it of value.
Hohlweg[15]	..	..	30	9	Ten cases were chronic passive congestion.
v. Frey[16]	..	..	26	14	Considering only those positive with more than 0.1 gm. sugar in urine.
Churchman[17]	38	9	12	10	Considers test unsatisfactory since neither a positive nor negative finding is conclusive.
Falk & Saxl[18]	..	..	351	259	Collected from literature.
Bruining[19]	..	..	30	27	Considered the test of value.

In reading the reports of most of these workers, difficulty is experienced in arriving at a conclusion concerning the value of the test in individual cases, since detailed clinical data and autopsy findings are not given. It appears, however, that the test is far from satisfactory and that much reliance from either a diagnostic or prognostic standpoint cannot be placed in its findings.

BAUER'S GALACTOSE TEST.

In 1906 Bauer[20] introduced galactose as a liver test. He administered 40 gm. in 400 to 500 cc. of tea on an empty stomach and determined quantitatively the amount of sugar present in the urine voided in the next 4 to 5 hours. In severe catarrhal jaundice large amounts of galactose appeared in the urine, but as the condition improved the amount in the urine quickly diminished. The amount recovered was greater in catarrhal jaundice than in jaundice due to other causes (gall stones, cancer). He considers it a test of liver function in catarrhal jaundice. Bondi and König,[21] Riess and Jehn,[22] and Hirose[23] confirmed this, believing it to be of importance in this connection.

The work of Falk and Saxl,[24] v. Frey[25] and Hirose[24] shows that the results of the test are very inconstant in diseases of the liver other than catarrhal jaundice.

In general, the shortcomings and disadvantages of carbohydrate tests, as they have been utilized, might be summarized as follows: (1) The use of arbitrary amounts of sugar without consideration of the normal tolerance of the individual patient; (2) the difficulty of keeping the patient on a carbohydrate free or a carbohydrate constant diet at the time of the test; (3) the practical difficulties of administration (nausea, vomiting, diarrhœa); (4) the disregard of such complications as portal obstruction, autonomic nervous derangement and disturbances of internal secretions influencing carbohydrate metabolism.*

UREA, AMINO ACID, AND AMMONIA NITROGEN.

Glaessner[25] showed in 1907 that in most instances of liver disease an unusually high excretion of amino acid N occurred and that the ratio of amino N to total N was also increased. Whereas normally he found the amino N constitutes only 0.2 to 0.4 per cent,† in pathological conditions of the liver it is decidedly higher, *e. g.*, secondary cancer of the liver 12.4 to 16.2 per cent, catarrhal jaundice 4.5 per cent, chronic alcoholic and fatty liver 6.9 to 8.33 per cent, luetic hepatitis 4.1 per cent, cirrhosis 3 to 13.4 per cent and in phosphorus liver 6 per cent. Falk and Hesky[26] showed that the urine of pregnant women contained increased amino acid N in 75 per cent of pregnancies. This they attributed to liver injury associated with pregnancy.

Falk and Saxl[24] have attempted further to follow the peptid N as well, determining both the amino and peptid N after feeding various nitrogenous foodstuffs to patients with normal and diseased livers. On feeding glycocoll to patients, they learned that diseased livers could not convert this amino acid into urea, but that it was excreted in part unchanged in

* Strauss (Deutsche med. Wchnschr., 1903, xxxix, 1780), presents further evidence to establish the value of the levulose test, and insists upon the adoption of a constant amount of galactose (30 gm.) in utilizing Bauer's test in order that the finding of the other tests may be compared.

† Henriques[28] using Sorensen's formol titration method, states that the amino acid N in man on an ordinary mixed diet constitutes 2 per cent of the total N. Levene and Van Slyke[27] place the normal amino acid content of urine as 1 per cent to 2.8 per cent of the total N.‡

‡ Kober (J. Am. Chem. Soc., 1913, xxxv, 1567), utilizing his new method for determining amino N places the amino N at 2.7 to 3.1 of the total N.

the urine. The peptid nitrogen they found increased when the amino N was high.

In a second communication these authors divided the liver cases into four groups, giving the results as indicated in the literature.

Group I.—Comprising tumors, sarcoma, carcinoma, leukæmia, amyloid disease, and chronic passive congestion. Normal amino and urea N values are encountered in sarcoma, leukæmia, amyloid disease, and chronic passive congestion (Stadelmann). In carcinoma the urea N may be reduced while the amino N per cent is high.

Group II.—Consisting of various intoxications, *e. g.*: chloroform, phosphorus and alcohol; and febrile conditions, such as typhoid, scarlet fever, and pneumonia. The febrile diseases are associated with only slight diminution of urea N, while in the other conditions—phosphorus poisoning, for instance, the decrease is somewhat greater, 74 to 86 per cent (Münzer), 55 to 85 per cent (Sjöqvist) of the total N instead of the normal 91 per cent.

Group III (Icterus).—In 27 cases of catarrhal jaundice and cholelithiasis there was no appreciable reduction of urea N which remained 80 to 87 per cent of the total N (von Noorden), 85 per cent (Mörner and Sjöqvist). The amino N is occasionally increased 8.1 per cent (Mörner and Sjöqvist), 4.9 to 9.5 per cent (von Noorden). The amino and peptid N is increased 4.5 per cent (Glaessner), 4.6 per cent (Falk and Saxl).

Group IV (Cirrhosis).—The urea N is slightly decreased. The amino N is invariably increased to more than 0.5 gm. according to v. Frey. This he considers of tremendous importance, since only rarely is so high an amino acid N output found in other diseases of the liver. The ammonia N is also constantly increased.

There is found in severe liver involvement, therefore, increase in the amino acid, peptid and NH_3N at the expense of urea N.*

Urobilinogen.

As early as 1892 the presence of urobilinogen in the urine was considered as indicative of disease of the liver by v. Jaksch.[29] The test consists simply in the qualitative determination of the presence of urobilinogen in the urine. Since Neubauer's[30] demonstration that Ehrlich's[31] p. dimethyl-amino-benzaldehyde test given by the urine in certain diseases is really a test for urobilinogen, this test is utilized. A few crystals of p. dimethyl-amino-benzaldehyd are added to a few cubic centimeters of urine, the mixture is shaken, and made definitely acid with HCl or acetic acid, whereupon an intense red color develops if urobilinogen be present.

Urobilinogen does not occur in health, the cycle in relation to bile pigments being as follows: Bile pigments are converted in the intestine into urobilinogen, which is absorbed, carried to the liver, and re-converted into ordinary bile pigments. The diseased liver cells having lost, to a greater or less extent, the capacity for reconversion, the urobilinogen, after absorption, continues to circulate and eventually finds its way into the urine. Urobilinogenuria, therefore, indicates functional incapacity of liver cells.

Münzer[32] states that urobilinogen appears in the urine in atrophic cirrhosis; Münzer and Bloch,[33] and also Fischer,[34] find that it appears in acute catarrhal jaundice prior to the appearance of the icterus, and in acute diseases associated with liver involvement (typhoid and pneumonia with acute hepatic parenchymatous changes). According to Münzer, urobilinogenuria is not present in pseudo-cirrhosis (Pick's disease), or in chronic passive congestion of the liver, where true liver disease is absent. He, therefore, considers the test of importance in differentiating between atrophic cirrhosis, on the one hand, and chronic passive congestion or pseudo-cirrhosis, on the other. Bauer,[35] however, states that it occurs in almost all diseases of the liver.

It is purely a qualitative test for the existence of liver disease. It probably has the advantage of specificity, since to no other cells has been ascribed the ability to convert urobilinogen into the ordinary bile pigments. From their own work and from a study of the literature, Falk and Saxl[24] conclude that urobilin excretion occurs in very slight injury to the liver, in which it is impossible to detect decreased carbohydrate tolerance or the N partition changes characteristic of insufficiency.

Fibrinogen.

Doyon and Kareff,[36] and Nolf[37] and his school showed that the extirpation of the liver was followed by the rapid disappearance of fibrinogen from the blood. In the report of an occasional experiment on animals, Doyon and his co-workers,[38 39 40] showed that decreased fibrinogen content occurred after chloroform poisoning, and Corin and Ansiaux[41] and Jacoby that it occurred also after phosphorus poisoning.

According to Whipple and Hurwitz,[42] fibrinogen normally exists in plasma of dogs in amounts varying between 0.2 to 0.5 gm. per 100 cc. blood. With the occurrence of liver injury produced by chloroform poisoning it decreases, falling at the time of injury and returning to above normal during the repair which rapidly follows. It may be present in such small amounts that hæmorrhage or hæmorrhagic tendency results, the clots being too soft to check bleeding.

In cases of acute hepatic disease (chloroform poisoning) in dogs and human beings it may fall to 0.048 to 0.034 gm. per 100 cc. blood, in chronic liver cirrhosis (Whipple[42]) to 0.05 gm. or even lower. This decrease is not constant. A high fibrinogen content may exist in the presence of definite liver disease. However, when the content is low it is of grave prognostic import.

The test is made as follows: 25 or 50 cc. of clear plasma obtained by centrifugalizing the blood, which has been received into oxalate solution, is heated in a water bath at

* Excellent reviews of this whole subject are given by v. Frey[16] and by Falk and Saxl.[24]

59° C. for 20 to 30 minutes. Fibrinogen is thrown out as a white flocculent precipitate, is collected on a Gooch crucible, washed with H_2O, alcohol and ether, dried and weighed.

LIPASE.

The amount of lipase in the blood has been shown to be markedly increased in certain diseases of the liver (Whipple, Mason & Peightal).[44] They utilized Loevenhart's[45] method of determining lipase, which is done according to the following technic: Four tubes are prepared, each containing 1 cc. of plasma, or serum, diluted with 4 cc. of distilled H_2O and to this is added 0.3 cc. toluol to prevent bacterial infection. To two of the tubes is added 0.26 cc. of ethyl butyrate, the other two serving as controls. After shaking, the tubes are stoppered and placed in an incubator at 38° C. for 18 to 24 hours, then cooled in water and to each is added three drops of azolitmin as an indicator. They are then titrated in pairs to neutrality, the controls with 1/10 N acid, the other with 1/10 N alkali. The controls with this indicator show the blood alkalinity to be 0.1 cc. of N/10 acid, while the butyrate tubes show an acidity of 0.1 to 1.2 cc. of N/10 alkali. The lipolytic activity of normal blood or serum expressed in terms of N/10 HCl is, therefore, 0.2 to 0.3 cc.

Experimental injury to the liver resulting from chloroform, phosphorus, hydrazine, etc., always produces an increase in plasma lipase to from 2 to 8 times the normal. After chloroform anæsthesia of 1 to 2 hours' duration, in dogs the plasma lipase increases to 1 to 2 cc. N/10 acid. This increase occurs during the first few hours after anæsthesia, lasts two to three days, and then slowly decreases as repair is established, finally reaching normal again on complete recovery. If the animal be fatally poisoned, the lipase remains high until death on the fourth or fifth day.

The test has been applied clinically by Whipple in a limited number of cases. A case of eclampsia showed a very high plasma lipolytic activity and at death showed hæmorrhagic portal liver necroses. Pneumonia, peritonitis, leukæmia, and various infections show an increased lipase, at times more than double the normal. Early stages of liver cirrhosis show a high lipase while late stages may, unless complicated with some liver necrosis, show a low lipase. A normal content was found in pernicious vomiting, uræmia with convulsions, in jaundice and obstructive jaundice of months' duration.

GHEDINI'S FERMENT TEST.

Within the current year Ghedini[46] has proposed a new test which is based upon the presence in the blood serum of a ferment which he claims arises in the liver cells and which is capable of converting glycogen into maltose, isomaltose, and glucose. In short, this must be a test for two blood ferments, diastase and maltase. Diastase varies tremendously in normal sera, consequently, *a priori*, this in itself would vitiate the test. Sufficient evidence has not been presented to establish the liver as the sole source of the ferment or ferments involved.

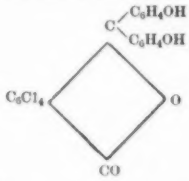
GENERAL CRITICISM OF FUNCTIONAL TESTS.

With the exception of the urobilinogen test, none of these deal with functions which can be said with certainty to be specific of the liver, since the muscles play a large rôle in carbohydrate metabolism, and the kidney, according to recent work of Van Slyke,[47] possibly participates in the conversion of amino acids into urea. The results of the tests seem to be inconstant, with the possible exception of the urobilinogen test, which is positive in most mild diseases of the liver. However, it only indicates the existence of liver injury and gives no conception of its extent.

Technical difficulties are encountered in some of the tests. The administration of large amounts of carbohydrates leads to nausea, vomiting, and diarrhœa, all of which vitiate the test. Considerable chemical training and equipment are necessary to carry on the work in relation to the nitrogenous metabolism, while large quantities of blood (50 cc.) are necessary for the fibrinogen studies.

Since the tests most used clinically have lacked a quantitative side they have proven of much less value from the standpoint of prognosis than similar studies in relation to the kidney. They have indicated only the presence of disease, but not the extent of involvement. Furthermore, the lack of clinical and autopsy data in the various publications detracts from their value, since one cannot form conclusions concerning the value of the findings in the individual cases.

PHENOL-TETRACHLOR-PHTHALEIN.



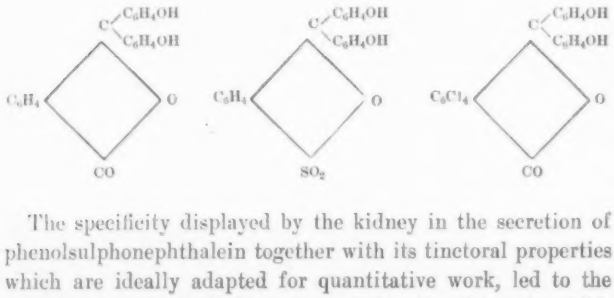
This compound was first prepared by Orndoff * and Black,[48] of Cornell University, in 1908. Its pharmacological properties were studied by Abel and Rowntree[49] in 1909. "Its physical and chemical properties present such similarities to those of phenolphthalein that one is surprised to learn that there are certain well-marked pharmacological differences between them. Like phenolphthalein, it is an odorless, tasteless, crystalline compound, insoluble in water and forming deeply colored hydrolizable salts with alkalies. Its ionization constant has not yet been determined, but its avidity as an acid cannot be far removed from that of phenolphthalein, inasmuch as solutions of its salts (Na or K) are promptly decolorized on the addition of serums or by contact with animal tissues. In this respect its salts differ in no way from those of phenolphthalein. The two compounds have, on the whole, a very similar pharmacological action."

* It is with great pleasure that we take this opportunity of expressing our gratitude to Professor Orndoff for his kind response to our repeated requests for the phenol-tetrachlor-phthalein used in this and other studies.

From the pharmacological studies of Abel and Rowntree[4] it was learned that phenol-tetrachlor-phthalein itself was non-irritant locally, but that solutions of its alkali salts administered subcutaneously act as decided irritants unless they are highly diluted since "such salts formed as they are by neutralizing a very weak acid with a very strong base are strongly hydrolyzed in aqueous solution and hence act upon the tissues like solutions of caustic soda." It was further learned in animal experimentation that the tetrachlor body has a very low toxicity; that it exercises no hemolytic influence; that it does not affect the coagulability of the blood; that, associated with its rapid intravenous injection, there occurs a fleeting drop of blood pressure which is followed by a slight increase (15 to 25 mm. Hg.) lasting 10 to 20 minutes, provided large injections are given; that it possesses no bactericidal or antiseptic properties; that it does not influence the rate of flow of either pancreatic juice or bile; that it appears in the bile first as a conjugated body and later in its free form, that in moderate quantities, following its subcutaneous administration in olive oil or its intravenous injection as a disodium salt, it escapes from the body only in the bile, the amount that passes into the intestines in other secretions being so minute that it can barely be detected; that a small amount is re-absorbed from the large intestine and that administered in a dose of 0.4 gm. in olive oil subcutaneously it exhibits laxative properties.

Further work showed that clinically the drug administered in olive oil displayed definite laxative characteristics, but that its low solubility in oil, necessitating a large bulky injection, stood in the way of its general adoption in this connection.

The effect of slight changes in the chemical formula of some of these phthaleins upon their pharmacological behavior, especially as to the channel of their excretion, is exceedingly interesting. Phenolphthalein given in moderate quantities subcutaneously in oil or intravenously as the disodium salt is excreted both in the urine and in the bile. The replacement of the CO by an SO_2 group yields phenolsulphonephthalein for which the kidney is the chief organ of excretion, while the substitution of the hydrogen atoms of the phthalein radical of phenolphthalein by four chlorine atoms gives phenol-tetrachlor-phthalein, the excretion of which is specific to the liver.



The specificity displayed by the kidney in the secretion of phenolsulphonephthalein together with its tinctoral properties which are ideally adapted for quantitative work, led to the introduction of this body as a renal functional test in which connection it has proven of the greatest value prognostically and diagnostically, and from the standpoint of treatment.

The striking specificity displayed by the liver towards the excretion of this dye suggested to one of us (R.) the possibility of utilizing the drug as a functional liver test, the underlying principle involved being identically that concerned in the phenolsulphonephthalein test of kidney function, *e. g.*, the specific excretion of a dye by a single organ and the decreased capacity for its excretion consequent upon lowered function resulting from disease.

With this idea in view the normal quantitative excretion was investigated and determined in rabbits to be from 30 to 45 per cent of the amount injected. The possibility of utilizing tetrachlorphthalein as a test for liver function was suggested to Dr. G. H. Whipple, who very kindly applied the test to a series of dogs suffering from experimental liver lesions. These preliminary tests showed a marked decreased excretion to exist in disease. A simultaneous clinical and experimental study has been carried on, the results of the experimental work being reported in this number of the BULLETIN by Whipple, Peightal and Clark.

METHOD OF PREPARING SOLUTION FOR INJECTION.

The preparation injected is an aqueous solution of the disodium salt prepared as follows:

2.5 gm. of phenol-tetrachlor-phthalein are placed in a 200 cc. Erlenmeyer flask with 5 cc. of 2/N NaOH solution and 45 cc. of freshly distilled water. This is boiled for 20 minutes under a reflux condenser. The solution is filtered into a 100 cc. flask, when it is ready for use. This gives approximately a 5 per cent solution which is almost isotonic with the blood.

The solution is an intense purplish red color. It will not keep for more than a few days since the phthalein is precipitated by CO_2 from the air. Even when CO_2 is excluded some precipitation still occurs from the loss of the alkali through union with the silicates of the glass container. In the event of only a small amount of precipitate, sterile filtration can be carried out and the resulting solution used for injection, provided a fresh standard for comparison is prepared.

METHOD OF ADMINISTRATION.

Arbitrarily 8 cc. of this solution, approximately 400 mg. of tetrachlor-phthalein has been selected. This amount is sufficient to give a most intense purplish red color to 20 litres of water. Its administration in health is never followed by the appearance of the dye in the urine,* and this amount insures in health an intense color in the final preparation of the fæces which is used for the quantitative determination.

The dye is administered intravenously by gravity with antiseptic and aseptic precautions and with the usual intravenous technic. The funnel and system are filled with freshly

* In health the dye has been recovered in the urine of a normal patient following injection of 0.5 gm.

distilled water and after the flow is well established the phthalein solution is added. Fifty to 100 cc. of water are used and the phthalein solution is washed in with freshly distilled water until the fluid entering the vein is colorless. Ten to 15 minutes are required for its administration. Physiological salt solution may be preferable to distilled water for use in this injection.

Collection of Materials for Study.

Active purgation is instituted prior to administration of the dye and throughout the time of observation, usually by means of compound cathartic pills. The stools are collected for 48 hours, the urine for 24 hours. In the event of little or no fæces being obtained enemata are used, but unless the normal amount of dye is recovered, the test must be discarded, since low findings under these conditions could not be accepted.

Method of Determining the Amount of Phthalein in Stools.

The total 48 hours fæces are placed in a 2 L. bottle and diluted with water to 1 or 1.5 L. depending on their amount. This is placed in a shaking machine for from 5 to 20 minutes. Without allowing time for sedimentation 1/10 of the total is placed in a 1 L. flask and to this is added 5 cc. of 40 per cent NaOH which causes the mixture to take on a dirty red color. Dilution is made with water to 1 L. A stopper is inserted and the mixture thoroughly shaken. One hundred cubic centimeters of this preparation is placed in a 200 cc. flask, 5 cc. of saturated basic lead acetate added, resulting in a decolorization of the mixture and the throwing out of a heavy lead precipitate which carries down all of the pigments, leaving a clear colorless supernatant fluid. Five cubic centimeters of 40 per cent NaOH are added; this again elicits the red phthalein color, but does not redissolve the other lead pigment combinations. In certain instances 5 cc. of NaOH at this point are not sufficient to elicit the maximum intensity of red and more should be added until the maximum is reached, but not sufficient to free the other pigments from their insoluble lead combination. The contents of the flask are made up to 200 cc., shaken, and a small part filtered off, or the solution is allowed to stand five minutes, when in many cases a clear red supernatant fluid ready for estimation can be decanted off. This solution is compared in the Rowntree and Geraghty modification of the Autenrieth Königsberger colorimeter with a 20 mg. to a litre solution of disodium salt of tetrachlor-phthalein (*e. g.*, 0.4 cc. of the original solution to 1 L. plus sufficient NaOH to insure maximum color). With these dilutions the amount of dye present is indicated directly in per cent.

When the amount recovered is below normal, it is advisable to add 2 to 3 cc. more alkali to the 200 cc. preparation and redetermine, thus insuring that the maximum color has been elicited. The addition of large quantities of alkalies is undesirable, since it sets free the other pigments, rendering the solution yellowish red instead of purplish red.

Not more than ten minutes are required to carry out the test after the fæces are removed from the shaker.

Where difficulty is experienced on account of the quality of the color, the following procedure, which may prove of some value in certain instances, may be utilized. After the addition of about 10 cc. of 40 per cent sodium hydroxide, the fæces are made up with water to one litre. To 1/10 of this is added 5 cc. of sodium hydroxide and water up to one litre. Of this 100 cc. is placed in a 200 cc. flask and to it 5 to 10 cc. or more, of a calcium chloride mixture * is added until the best quality of color is elicited. Dilution is made to 200 cc., the mixture is allowed to stand from one-half to 24 hours, a small amount of the supernatant fluid is filtered off, and read against the standard.

In our earlier methods an attempt was made simply to dilute the fæces, filter off and determine the amount present, but quantitative determination is usually impossible in this way on account of the large amount of coloring matter present in the mixture. Later basic lead acetate was used and the precipitate collected on a filter, extracted repeatedly with hot alkaline alcohol and the alcohol diluted to the proper extent, alkali added and determinations made. This method was time consuming, required much alcohol, and the extraction of tetrachlor-phthalein was never complete. Finally the method presented above was devised.

Accuracy of Method.

A number of procedures were utilized to determine the degree of accuracy of the method and to determine what part the personal equation plays in regard to it.

Is the total dye substance recovered? Accurately measured amounts of the original solution of the disodium salt were added to the fæces, thoroughly mixed by one member of our group, and determined by another who was unfamiliar with the amount added. In certain instances independent readings were made by the entire group. The results are as follows:

Table I.

Number	Amount added	Amount recovered B	R	H
I	25 %	.....	23.5%	.....
II	12.5%	.....	12 %	.....
III	12.5%	12 %	12.5%	12.5%
IV	9.4%	8 %	10 %	10 %
V	25 %	23.5%	23 %	22.5%
VI	18.8%	17 %	19 %	17 %

Since only an aliquot portion of the fæces is used for the determination, the necessity of justifying this procedure seemed advisable. This was done as follows: The technic was performed on several fractions by one and readings made on the final solution by the three members of the group. The results in per cent are shown in Table II.

* $CaCl_2$ 90 gm.
Conc. NH_4OH 10 cc.
Water 50 cc.

TABLE II.

Number	B	R	H
I	16 %	14 %	16 %
II	16 %	15 %	17 %
III	17.5%	15.5%	16 %
IV	16 %	14 %	15.5%
V	17 %	14.5%	17 %
VI	16.5%	15 %	17 %
VII	16 %	13.5%	15 %

From the table it will be seen that differences in reading the same solution may occur where various individuals are making the readings, R. reading constantly lower than B. or H., the difference, however, never being greater than 2.5 per cent.

Simultaneously it is seen that slight differences occur in different fractions, as evidenced by III and VII, the latter being lower according to all three observers. Here again, however, the difference is only slight.

It was also considered advisable to check the readings on various fractions at the point where lead acetate is added. The following table shows a series of readings of different fractions:

TABLE III.

Number	R	B	H
I	29 %	30 %	29 %
II	28.5%	30 %	28 %
III	29 %	29.5%	28.5%
IV	28.5%	29.5%	28 %
V	28 %	29.5%	27 %
VI	28.5%	29 %	30 %
VII	29.5%	29.5%	29.5%

No destruction of the dye occurs after the stools are collected, so that immediate determination is not necessary. The following table indicates the stability of the drug.

TABLE IV.

Patient	First determination	Time elapsing	Second determination
P.	54%	1 week	55 %
M.	18%	1 "	20 %
L.	47%	5 "	45 %
McC.	31%	5 "	33 %
S.	28%	7 "	32 %
G.	33%	12 "	33 %
B.	26%	15 "	24.5%
A.	26%	19 "	23 %

No preservatives were used so that decomposition of the dye does not apparently result from bacterial infection. Slight differences in the readings can be explained possibly by differences in the standards used for comparison, since in the first instance each test was made from the standard prepared from the solution injected, whereas all of the later readings were made against a single freshly prepared solution.

It is evident, therefore, that the test, though not absolutely quantitative, gives reliable results approximately correct, and that dependence can ordinarily be placed upon its findings, errors of more than 5 per cent being rarely encountered. Difficulties may arise at times, as will be seen later.

UNDESIRABLE FEATURES OF THE TEST.

The collection of total fæces for 48 hours is the most difficult part of the technic to control. It certainly constitutes an objectionable feature of the test. Simple as it may seem, even in the best institutions, except under special provisions, it is difficult to find a corps of attendants capable of carrying out proper collections. Despite all precautions, in four instances some of the fæces was lost in collecting. In these cases no drug at all, or a small amount only (at most 10 per cent), was recovered, the patients showing neither signs nor symptoms of liver involvement. Repetitions within a week in each instance revealed a normal, or practically normal excretion. Careful questioning of patients and attendants showed in one or two instances that losses undoubtedly had occurred.

In two other cases, both myocardial decompensation, repetition of the test within a week showed a tremendous increase in the dye output. Hesitation is felt in claiming that the increase was entirely due to improvement in liver function, although both cases were *in extremis* at the time of the first test and clinically in fair condition at the time of repetition. Until other such cases are encountered where more certainty exists in relation to total collection, losses in collection cannot be excluded as the cause of the low output in the first instance.

The collection over 48 hours has a second objectionable feature. The excretion of the drug occupies but 12 to 18 hours in health, consequently 48-hour collections are apt not to show minor grades of injury so well, since the continuous secretion of smaller amounts of the dye over longer periods, 24 to 36 to 48 hours, may bring the total output close to normal.

The quality of red color obtained in certain instances constitutes the third objectionable feature of the test. This objection is minimized by the method now utilized. However, with the best technic the color finally obtained may in a considerable proportion of cases (10-20 per cent) exhibit yellowish red or brownish red qualities instead of the purple red desired. Such determinations are less accurate, but only in rare instances does the color constitute a very serious difficulty.

In myocardial insufficiency with feeble circulation localized thrombosis of the veins at the point of injection may occur. This has been encountered in ten instances in this series. Slight local pain together with resistance offered to the palpating finger was noted. Thrombosis, however, was not encountered except in myocardial insufficiency, marked anæmia, or where advanced phlebosclerosis was present. The introduction of large quantities of fluids is inadvisable in the presence of a weak myocardium and consequently in this connection great dilution cannot be utilized.

The fate of the unrecovered portion is unknown. Apparently, however, no destruction of the drug occurs after its entrance into the intestinal tract, since the amount of drug recovered in a dog's fæces corresponds closely to that recovered directly from a permanent biliary fistula following the same dosage.

The test is inapplicable where obstruction to the biliary passages exists and is, therefore, limited in its application.

TABLE V. NORMAL CONTROLS.

No.	Name.	No.	Diagnosis.	Age—yrs.	Date—1913.	Per cent. in faeces.	Urine.	Remarks.
1.	S. H.		Normal	27	1-28	30	..	
2.	A. B.		Normal	25	1-28	30	..	
3.	B. E.	87296	Convalescent typhoid	22	2-15	42	..	Temperature normal for 1 week.
4.	C. McC.	87584	Splanchnoptosis	32	2-22	39	..	
5.	F. B.	87617	Orthostatic albuminuria	17	2-22	31	..	
6.	A. L.	87810	Neurasthenia	45	2-28	52	..	
7.	A. A.	87907	Diabetes	48	3-5	40	..	
8.	M. M. C.	87809	*Chronic tuberculosis	44	3-19	*22-33	..	Afebrile 1 week; earlier 22%.
9.	J. M.	88157	Neurasthenia	60	3-19	52	..	
10.	D. W.		Convalescent pneumonia	27	6-10	24	..	Afebrile 1 week.
11.	L.		Convalescent malaria	22	6-10	48	..	Afebrile 6 days.
12.	L. F.	89350	Rheumatic fever convalescent	24	5-29	30	..	Afebrile 1 week.
13.	C.		Pulmonary tuberculosis	..	6-13	39	..	Afebrile.
14.	W.		Fracture of femur	..	6-17	42	..	
15.	K.		Fracture of femur	..	6-17	34	..	
16.	A.		Gunshot wound	..	6-20	40	(1)	
17.	B.		Fracture	..	6-20	35	(2)	500 mg. injected.
18.	M.		Gonorrhœal arthritis	..	6-20	34	..	

(1) + Slight. (2) + Considerable.

TABLE VI. FEBRILE CASES.

No.	Name.	No.	Diagnosis.	Age—yrs.	Date—1913.	Per cent. in stool.	Urine.	Remarks.
19.	G. S.	87326	Pulmonary tuberculosis; pleurisy with effusion	43	2-7	43	..	99-102°F.
20.	J. F.	86802	Pulmonary tuberculosis; pleurisy with effusion	34	2-7	40	..	99-102°F. for 2½ months.
21.	I. B.	87671	Typhoid fever	14	2-28	27	..	15th day of fever.
22.	S. T.	88038	Acute lobar pneumonia	18	3-10	39	..	Fifth day of disease temperature 104-105°F.
23.	A. B.	87794	Acute tubercular pleurisy with effusion	28	3-19	43	..	Continuous fever 104°F. for over 1 month.
24.	B. K.		Acute rheumatic endocarditis	30	3-19	40	..	Temperature 103°F. for 1 month.

TABLE VII. ANAEMIA.

No.	Name.	No.	Diagnosis.	Age—yrs.	Date—1913.	Per cent. in stool.	Urine.	Hb.	R. B. C.
25.	C. R.	88350	Hypernephroma	56	3-29	41	..	28	1,600,000
26.	O. K.	88692	Secondary anaemia; hemorrhoids	21	4-24	35	..	21	2,680,000
27.	J. S.	88675	Pernicious anaemia	20	4-23	18-20	..	31	1,700,000
28.	W. S.	88825	Secondary anaemia	45	4-27	28	..	23	2,700,000
29.	H.		Gastric carcinoma	38	5-24 6-10	14 24	..	20	2,300,000
30.	L. F.		Gastric carcinoma	39	6-10	20	..	22	2,800,000

The possibility of utilizing the time required for the disappearance of the drug from the blood after its administration is being tried in the hope that some of these difficulties may be surmounted.

The Results of the Test.

Eighty determinations of liver function have been made in 67 patients. The series contains normal controls and various types of liver injury.* The test was applied in 24 cases in which the livers were believed to be normal. The amount of drug excreted varied from 30 to 52 per cent, as will be seen in Tables V and VI. In one constipated patient only 24 per cent was recovered.

Cases in Table VI are considered as normal controls, since the excretion is normal with one exception, a case of typhoid (third week) in which focal necrosis cannot be excluded. The average output in afebrile normal cases was 37.4 per cent, while that in the febrile class was 39 per cent. The lower limit of normal is, therefore, considered 30 per cent for 48 hours.†

Dogs with biliary fistulæ furnished some interesting data in relation to the rate of normal excretion of the drug. It appears in the bile in its free form regularly within 10 to 15 minutes following the intravenous administration of 100 to 200 mg. The same time is required in human beings, since, in the one case studied after operation, the drug was present in the bile after 15 minutes. The largest amount recovered from dogs was 55 per cent. The maximum excretion is quickly reached, since in one instance 27 per cent was recovered during the first 6 hours. In health excretion is complete within 16 to 20 hours.

The dye output is independent of the quantity of the bile excreted. Following the injection of the same amount of phthalein into the same dog more dye was recovered in 8 cc. in one instance than in 80 cc. of bile in another instance. This is analogous to the independence between the amount of sulphonephthalein excreted and the quantity of urine.

Influence of Fever.

Pyrexia *per se* has no effect upon liver function, as will be seen from Table VI. Case Q. T. was an acute febrile condition with temperature 104° to 105° F. at the time of the test, and case A. B., a chronic one, the temperature reaching 104° F. every day for over a month, yet both showed a normal phthalein output. Case I. B. is discussed above.

Influence of Anæmia.

In severe grades of secondary anæmia a normal, or practically normal, output may be encountered, as in cases O. K. and W. S., Table VII. From these cases it is justifiable to conclude that secondary anæmias of slow development must be of an extreme grade before liver function is affected. Experimental evidence indicates, however, that acutely developing severe secondary anæmia does influence the phthalein output, as will be seen from the following protocol.

Female Dog. 18.5 Kg.

5- 3-13. Excreted 35 per cent tetrachlorphthalein.
5- 9-13. Bled 640 cc. The following morning Hb. 64 per cent. R. B. C., 4,800,000. Injected 200 mg. phthalein.
5-12-13. Hb. 75 per cent. Phthalein recovered 41 per cent.
5-13-13. Bled 600 cc.—ether anæsthesia. The following morning Hb. 48 per cent. R. B. C., 3,840,000. Phthalein injected.
5-16-13. Hb. 50 per cent. Phthalein recovered 30 per cent.
5-18-13. Bled 650 cc.—ether anæsthesia. The following morning Hb. 42 per cent.
5-20-13. Hb. 42 per cent. Phthalein recovered 15 per cent.*
5-26-13. Bled 600 cc. The following morning Hb. 30 per cent. R. B. C., 2,400,000.
5-29-13. Phthalein recovered 13 per cent.
6-12-13. Phthalein recovered 27 per cent.
7- 7-13. Hb. 90 per cent. R. B. C., 5,586,000. Phthalein injected.
7- 9-13. Phthalein recovered 39 per cent.

A second dog responded in the same way, the phthalein decreasing from 50 per cent to 10 per cent, at which time the Hb. was 30 per cent and the R. B. C. below 2,000,000. This dog, however, had been accidentally inoculated with sarcoma which was unexpectedly discovered from microscopical study of the liver, the dog dying on the table during the last bleeding.

There exists a striking analogy in the influence of secondary anæmia on liver and on kidney function, as will be seen by comparing the above protocols with experiments previously reported by Rowntree and Geraghty[51] on the influence of anæmia on kidney function.

The two cases of anæmia secondary to gastric carcinoma show decreased function. However, metastasis cannot be excluded in either instance. In severe phenyl hydrazine anæmia in dogs Whipple saw but little influence on liver function. In the single case of pernicious anæmia a decreased output was found.

From such a limited number of cases no conclusions can be drawn except that secondary anæmia must be of an extreme grade before the excretion of the dye is decreased. The experimental results suggest the necessity of more observation along these lines.

Hepatopathies.

Thirty-seven cases exhibiting signs or symptoms of abnormalities of the liver have been investigated, the series including enlargement dependent upon cardiac disease, cirrhosis, carcinoma, amœbic abscess, luetic hepatitis, cholecystitis, and enlargement associated with leukæmia. A detailed description of the history and clinical findings of each of these cases, together with the phthalein output, is appended. Table VIII shows the results of the test in these cases.

* We wish to express our thanks to Dr. Ernst Zueblin of the University of Maryland Hospital, to Drs. Boggs and Snowden of Bay View Hospital, and to the Medical and Surgical staffs of our hospital for the opportunity of studying the clinical material.

† 48-hour collections are necessary, since in several instances an appreciable amount of dye (8 to 10%) was recovered in the second 24 hours.

* 57 per cent of sulphonephthalein was recovered in two hours. This indicates only a slight reduction in kidney function.

TABLE VIII. PATHOLOGICAL LIVERS. HEPATOPATHIES.

No.	Name.	No.	Clinical diagnosis.	Age—yrs.	Date—1913.	Per cent. in stool.	Urine.	Remarks.
31.	C. H.	87440	Myocardial insufficiency; acute endocarditis; tuberculous polyserositis.	47	1–7 2–14 4–26	7 18 15	.. .. ..	Autopsy.
32.	T. A.	87515	Arteriosclerosis; chronic nephritis; mild myocardial insufficiency.	39	2–5	28	..	
33.	R. S.	87589	Myocardial insufficiency; arteriosclerosis	36	2–15	9	..	
34.	M. L.	87653	Myocardial insufficiency; mitral insufficiency and stenosis; adherent pericardium.	48	2–22	33	..	
35.	P. G.	87770	Myocardial insufficiency; aortic and mitral insufficiency.	23	2–28	42	..	
36.	C. G.	87747	Myocardial insufficiency; chronic bronchitis; emphysema; cirrhosis of liver?	66	2–28	33	..	
37.	R. B.	87855	Myocardial insufficiency; arteriosclerosis	45	3–5	8	..	Small specimen of stool.
38.	C. G.	88093	Myocardial insufficiency; myocarditis; acute endocarditis.	51	3–15	35	..	Autopsy.
39.	J. F.	88071	Myocardial insufficiency; mitral stenosis and insufficiency.	40	3–15	33	..	
40.	B. S.	88324	Myocardial insufficiency; mitral insufficiency and stenosis; aortic insufficiency.	27	3–24	33	..	Died 4–12. No autopsy.
41.	E. G.	88884	Myocardial insufficiency; dilated aortic arch; syphilis; chronic nephritis?	42	4–27	32	..	
42.	J. J.		Myocardial insufficiency; aortic insufficiency; dilated aortic arch; syphilis.	43	5–12 5–19	5* 24	.. ..	Autopsy 6–15–13.
43.	T. G.	89247	Arteriosclerosis; myocardial degeneration; chronic nephritis	52	5–17 5–24	30 28–30	.. ..	
44.	I. W.	89144	Myocardial insufficiency; arteriosclerosis	48	5–22	28	..	
45.	I. B.	89301	Miliary tuberculosis; tuberculous pericarditis (adherent); myocardial insufficiency.	26	5–27	21	..	Autopsy.
46.	S. R.	89417	Myocardial insufficiency.	33	5–24 6–3	7 33	.. ..	In severe cardiac break. In good clinical condition.
47.	R. P.	(1)	Myocardial insufficiency	..	4–28	35	..	
48.	M.		Myocardial insufficiency	40	6–13	20	..	In moderate cardiac decompensation.
49.	J. N.	88072	Cirrhosis of liver	52	3–10	23	..	Normal fibrinogen; clinically well, admitted only for test.
50.	D. M.	(2)	Cirrhosis of liver	56	3–26	6	..	Special precautions in collecting specimens.
51.	W. G.	(1)	Myocardial insufficiency; cirrhosis of liver	..	4–28	43	..	Died about 3 weeks later. No autopsy.
52.	K. Fischer	(3)	Carcinoma of stomach; metastasis to liver	48	3–19	8	1.5%	Autopsy.
53.	A. Hepburn	(2)	Carcinoma of gall bladder	67	4–22	20	..	
54.	E. S.	88424	Carcinoma of stomach with metastasis	33	3–31 4–8	6* 24	.. ..	Unsatisfactory specimen of stools.
55.	G. D.		Carcinoma of stomach with metastasis to liver	72	....	14	..	
56.	M. S.	87656	General abdominal carcinomatosis	40	3–5	39	..	
57.	J. T.	89781	Carcinoma of liver	45	6–20	7	(4)	Collection for 30 hours.
58.	M. B.	87983	Suspected amoebic abscess of liver	27	3–10	33	..	Explored; no abscess found.
59.	J. T.	(1)	Amoebic abscess of liver; drained	45?	4–28	23	..	
60.	F. R.	88825	Hepatitis; tuberculous peritonitis (?)	40	4–27	30	..	Unimproved on discharge.
61.	E. P.	88861	Splenomegaly; perihepatitis (?)	19	5–10	23	..	Discharged; condit'n unchanged.
62.	J. McC.	89082	Tuberculous peritonitis (?); syphilitic hepatitis (?); mitral insufficiency.	52	5–17 6–10	31 32	.. ..	
63.	J. K.	89439	Acute cholecystitis	56	5–29	28–30	..	Discharged; well.
64.	H. A.	89593	Tuberculous peritonitis (?); liver cirrhosis (?)	52	6–7	22	..	Discharged; condit'n unchanged.
65.	E. T.	(2)	Syphilis of liver; syphilitic endocarditis; mitral insufficiency.	33	4–22	18–22	..	
66.	G. S.	(2)	Syphilitic hepatitis (?); amyloid liver (?); pulmonary tuberculosis.	27	4–22	40	..	Autopsy.
67.	R. S. P.		Lymphatic leukaemia	..	6–13	55	..	

(1) University Hospital. (2) Bay View. (3) Surgical No. 31832. (4) +Slight.

Myocardial Insufficiency.

Eighteen of the hepatopathies were associated with some degree of myocardial insufficiency. In nine the phthalein output was normal. Five cases showed marked involvement of function as indicated by the test and in each instance the low output was associated with an extreme grade of broken compensation, whereas the break in the nine cases already mentioned was much less severe. In two instances a subsequent test a week later, at which time the cardiac condition was improved, revealed a marked increase in the phthalein excretion, although hesitation is felt, as intimated above, in ascribing this difference between the two findings entirely to restoration of cardiac compensation.

Of the other four cases, one with a 21 per cent output (I. B.) had miliary tuberculosis which affected the liver, one (M) in moderate decompensation excreted 20 per cent, while two others (T. A. and I. W.) were practically normal (28 per cent).

It is therefore evident that liver function as indicated by the test only becomes seriously affected in those cases in which the myocardial insufficiency is extreme. This is in striking analogy to the condition of renal function as indicated by sulphonephthalein,[32] for only in the cases of extreme cardiac decompensation or passive congestion, clinical or experimental, is the output of phthalein appreciably decreased. The first evidence of cardiac improvement is also associated with a prompt return of renal function to normal.

Cirrhosis of the Liver.

Two cases, typical clinically in every respect, have been studied. One, D. M., was *in extremis* at the time of the test, showing both toxic and obstructive symptoms. His output was only 6 per cent and no dye appeared in his urine. Death occurred two months later, but no autopsy was obtained. The other case, J. N., had been under observation for years. Six years earlier, he had 272 litres of ascitic fluid removed. For some years, however, he has been practically free of symptoms, carrying on his usual work. The patient considers himself well and exhibits but few residues of his former disease. The output, 23 per cent, is well in keeping with his present clinical condition.

Cirrhosis possibly existed in two other cases, W. G. and H. A. The former clinically looked like cirrhosis, but myocardial insufficiency was also present. A normal output was found. The patient died three weeks later, no autopsy being obtained. The existence of cirrhosis in the other patient, H. A., with a 22 per cent output, is very questionable.

The limited number of cases and the absence of autopsy data allow of no conclusions concerning the excretion of the drug in cirrhosis.

Carcinoma of the Liver.

Three verified cases of liver carcinoma showed a decreased function. In one, K. F., the output was only 6 per cent. This patient came to autopsy, the destruction of liver tissue being very marked, together with a uniform central necrosis involving about two-fifths of each lobule. The other patients showed 7 and 14 per cent excretion respectively, the liver involvement being marked on exploration. H., with cancer of the gall bladder, excreted 20 per cent. This patient is still living.

In one patient, E. S., suffering from carcinoma of the stomach, 24 per cent was recovered. No metastases were visible on exploration.

In one case of general abdominal carcinomatosis without liver involvement, M. S., there was no decrease in phthalein output.

Amœbic Abscess.

A week after the institution of drainage in a case of amœbic abscess of the liver (W. G.) the test was applied and 23 per cent recovered, the patient at this time being in a good clinical condition. A second suspected case showed a normal function. This case was explored by Professor Halsted and no abscess was found.

Miscellaneous Liver Cases.

Three of these cases were very similar clinically, exhibiting an unexplained ascites. Luetic hepatitis and tuberculous peritonitis were suspected. Two showed a normal function, while 22 per cent was recovered in the other case in which liver cirrhosis also had been suggested as a clinical diagnosis.

One case of cholecystitis showed a normal function. E. P., with a diagnosis of splenomegaly and perihepatitis, excreted 23 per cent, while from E. T., with luetic hepatitis and myocardial insufficiency 18 to 22 per cent was recovered.

G. S., in whom an amyloid and luetic liver was diagnosed clinically, had a normal excretion. The patient later died of his pulmonary tuberculosis and at autopsy the liver change present was chronic passive congestion with some atrophy.

The highest phthalein output in this entire series was encountered in a case of lymphatic leukæmia with enlarged liver.

Unfortunately it has been possible to apply this test only to a limited number of diseased conditions. There still remain a number of diseases, for instance uræmia, eclampsia, pernicious vomiting, septicæmia, peritonitis, and the like, associated with intoxication and depression of functional activity in which the results of the phenoltetrachlorphthalein test may be of interest prognostically.

The number of cases studied is too small to permit of far-reaching conclusions. However, the constant findings in health, the decreased output in liver disease, the analogy between the effect of anæmia and myocardial insufficiency upon kidney and liver function as indicated by the sulphonephthalein and the tetrachlorphthalein tests, the results of the test in experimental liver lesions, and the established value of sulphonephthalein, based upon the same principle as the test in kidney diseases, all indicate that the excretion of tetrachlorphthalein will be useful in the estimation of the functional capacity of the liver.

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APPENDIX.

(25.) No. 88350. C. R., age 56, male, colored.

Clinical diagnosis: Hypernephroma (rt. kidney). Metastases. Myocardial insufficiency. Chronic nephritis. Uremia. For eight months difficulty on urination with passage of blood. Much strangury. On admission: Extreme emaciation, slight dyspnœa. Heart enlarged with systolic blow. Liver dulness from fifth rib to 8 cm. below costal margin in right mamillary line where edge is indefinitely felt. Not tender. Below liver in right flank is felt a round tumor, probably kidney. No edema of feet. At time of test under rest in bed liver dulness had gone up to 2 cm. below costal margin. Blood examination: Hb., 28 per cent; R. B. C., 1,600,000. March 29, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 41 per cent. Death, April 4. *Autopsy:* 3901. Hypernephroma of kidney. Dilatation and hypertrophy of heart. Chronic passive congestion of lungs. Cloudy swelling of viscera, etc. Liver weighs 2300 gm. Liver. Microscopical section. The lobules show central congestion, pretty extensive hyaline necrosis, probably not over 48 hours old. The lobulation is regular.

(31.) No. 87440. C. H., age 49, male, colored.

Clinical diagnosis: Tuberculous polyserositis. Acute endocarditis. Weakness, loss of weight, shortness of breath, gradual swelling of legs and abdomen. On admission presented signs of a myocardial break. Liver, hard, firm, smooth, to umbilicus. R B. C., 3,000,000; Hb., 35 per cent. At time of second test conditions practically those shown at autopsy. Progressive emaciation, asthenia, anemia, persistent ascites. February 14, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 18 per cent. April 26, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 15 per cent. *Autopsy:* 3915. Tuberculous peritonitis, cloudy swelling of viscera, fibrous myocarditis, cardiac hypertrophy and dilatation, syphilitic aortitis. Liver weighs 2150 gm. The microscopical section shows subacute diffuse hepatitis with increase in connective tissue in all parts of each lobule and considerable deformity of liver architecture. Wandering cells of every type and scattered tubercles can be found. The liver cells show atrophy and degeneration as well as distortion.

(32.) No. 87515. T. A., age 39, male, white.

Clinical diagnosis: Arteriosclerosis, chronic nephritis. Symptoms for about one month: Palpitation, dizziness, smothering, sensations. At time of test, heart 14 cm. to left, 5 cm. to right. Systolic puff at apex. Aortic second sound loud. Liver dulness from fifth interspace to two fingers' breadths above costal margin in right mamillary line. Bases of lungs clear. Blood pressure, 200. Nothing to indicate any abnormality of liver except perhaps slight recent temporary chronic passive congestion of liver, not present at time of test. Blood examination: Hb., 90 per cent; R. B. C., 5,000,000. February 15, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 28 per cent. Discharged March 13, 1913. Improved.

(33.) No. 87589. R. S., age 36, male, colored.

Clinical diagnosis: Myocardial insufficiency. Arteriosclerosis. Pleurisy with effusion. Two previous admissions within past six months in badly broken compensation. At time of test, orthopnœa, fluid in right chest below angle of scapula. Edema of legs. Heart, 15 cm. to left, pulse rapid (110). Liver, firm, reaches about palm's breadth below costal margin. Tenderness over it. On discharge three weeks later, no dyspnœa, edema or ascites, but liver still a palm's breadth down, no tenderness. Impression: Evidently permanent induration of liver from prolonged decompensation with additional acute passive congestion at time of test. Blood examination: Hb., 60 per cent; R. B. C., 4,100,000. February 15, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 9 per cent. Discharged March 9, 1913. At no time any toxic symptoms to indicate hepatic insufficiency.

(34.) No. 87653. M. L., age 48, male, white.

Clinical diagnosis: Myocardial insufficiency, mitral insufficiency and stenosis, adherent pericardium. Acute polyarthritis 12 years ago. Bronchitis, dyspnœa on exertion, palpitation, and

precordial pain for past four winters. Comes in for these symptoms. Transient hemiplegia (embolus?) five weeks ago. At time of test, heart 12.5 cm. to left and 4.5 to right. Aortic diastolic, apical systolic and presystolic murmurs. Positive Broadbent. Border of heart dulness moves 4 cm. Lung descends slightly over cardiac flatness. No ascites or edema of extremities. Râles at lung bases. Liver felt ¾ cm. below costal margin in mamillary line but no definite edge. Impression: Perhaps slight permanent liver induration from prolonged chronic passive congestion. (No note of condition of liver on discharge.) Nothing to indicate marked chronic passive congestion of liver or fibrosis. Blood examination: Hb., 85 per cent; R. B. C., 4,400,000. February 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 33 per cent. Discharged March 17, 1913. Improved.

(35.) No. 87770. P. J., age 23, male, white.

Clinical diagnosis: Myocardial insufficiency. Aortic and mitral insufficiency. Right hydrothorax. Admitted eight months previous for acute polyarthritis. History of attack six years previously. Present complaints: For three days pains in legs, back, elbows, shoulders, fever and cough. At time of test, no dyspnœa or cyanosis. Right hydrothorax. Left lung clear to base. Heart 14 cm. to left and 4 cm. to right. Aortic diastolic and mitral systolic murmurs. Pulse 80. Liver dulness to costal margin; edge not felt. No edema of legs. No fever. Impression: No indication of chronic passive congestion of liver at time of test or of any chronic fibrosis. Blood examination: Hb., 84 per cent; R. B. C., 4,632,000. February 28, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 42 per cent. Discharged April 3, Improved.

(36.) No. 87747. C. G., age 66, male white.

Clinical diagnosis: Chronic bronchitis, emphysema. Cirrhosis of liver (?). Admitted in December, 1910. Diagnosis, chronic myocarditis. For six years shortness of breath on exertion. Winter cough. Pains in epigastrium since 1907, occasional nausea and vomiting. Findings on previous admission: Obesity, emphysema, chronic bronchitis, cardiac arrhythmia, no cardiac enlargement, no edema. Liver dulness from sixth rib above to 7 cm. below costal margin in mamillary line. Liver hard and not tender, edge not felt. On present admission: Chronic bronchitis, emphysema, cardiac dulness perhaps a little wide. Heart regular. No edema or ascites. Liver: Dulness to just below costal margin. Surface firm and hard, edge not felt. Impression: Liver pushed down by emphysema. Possible slight induration secondary to old myocardial condition. No definite evidence for cirrhosis. Blood examination: Hb., 87 per cent; R. B. C., 4,300,000. February 28, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 33 per cent. Discharged March 3, 1913. Improved. In good condition three weeks later.

(37.) No. 87855. R. B., age 45, male, colored.

Clinical diagnosis: Myocardial insufficiency. Arteriosclerosis. Chronic nephritis. For four years dyspnœa and cough. Six months before admission swelling of abdomen and legs. Nausea and vomiting two weeks. At time of test, dyspnœa, edema of lungs, heart 17.5 cm. to left and 4.5 to right. No murmurs. Liver four fingers' breadth below costal margin in right mamillary line. Firm, tenderness over it. Edema of ankles. On discharge no myocardial insufficiency. Ten days before liver dulness two fingers' breadth below costal margin; edge not felt. Impression: At time of test marked recent stasis in liver. Probably some chronic induration of liver from prolonged myocardial insufficiency. Blood examination: Hb., 80 per cent; R. B. C., 5,500,000. March 15, 1913, injected 380 mg. Amount in urine, 0. Amount in stools, 8 per cent. Small specimen stool.

(38.) No. 88093. C. G., age 51, male, white.

Clinical diagnosis: Myocardial insufficiency, myocarditis, acute endocarditis, pulmonary and renal infarctions. Thrombosis of left iliac veins. Indefinite spell of edema of feet two years ago. No symptoms until two weeks ago, when he had dyspnœa, pain in side, and swelling of legs. At time of test, moderate dyspnœa; no edema or ascites; liver indefinitely felt three fingers' breadth below costal margin. On admission four days before, edema of legs, dyspnœa and cyanosis. Heart 16 mm. to left and six to right. Liver from fifth interspace to 7 cm. below costal margin. Edge readily felt, rounded and firm, surface smooth. Subsequently developed an active endocarditis with fever, leucocytosis, infarctions. Died April 19. Impression: At time of test acute chronic passive congestion had practically cleared up. No evidence of chronic induration. Blood examination: Hb., 91 per cent; R. B. C., 4,300,000. March 15, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 35 per cent. Death. *Autopsy:* 3909. Thrombosis at site of injection. Liver 1900 gm. Surface slightly roughened. Typical nutmeg liver. Dark almost black central zones and yellow opaque looking partial zones. Liver. Microscopical section shows evidence of extreme passive congestion with central atrophy and fatty degeneration. There is some increase in connective tissue about the margins of the lobule, not, however, a definite cirrhosis.

(39.) No. 88071. J. F., age 40, male, white.

Clinical diagnosis: Myocardial insufficiency. Mitral stenosis and insufficiency. Acute and chronic bronchitis. Emphysema. Two previous admissions within past year with broken compensation and large tender liver. On admission March 10, 1913, dyspnœa, cyanosis, enlargement of heart 16 cm. to left and 5 cm. to right; cardiac murmurs; no edema of ankles; general bronchitis; no ascites. Liver dulness from fifth interspace to 4 cm. below costal margin where it is indefinitely felt. At time of test, only slight dyspnœa, no edema or ascites, slight cough. Liver felt 3 cm. below costal margin. Edge not distinct. Impression: Slight clearing chronic passive congestion. Nothing to indicate red atrophy of liver. Blood examination: Hb., 77 per cent; R. B. C., 5,200,000. March 15, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 33 per cent. Discharged much improved.

(40.) No. 88324. B. S., age 27, male, white.

Clinical diagnosis: Myocardial insufficiency. Mitral insufficiency and stenosis. Aortic insufficiency. Pulmonary infarctions. Acute polyarthritis seven years ago. Three months ago, cough, blood-tinged expectoration, dsypnœa, palpitation. Four to five weeks of swelling of legs. On admission: Enlarged heart with murmurs. Diffuse bronchitis and edema of lungs. Ascites, edema of legs. Dyspnœa. Liver edge indefinite, felt 3.5 cm. below costal margin. Tender over it. Test four days later: Condition essentially same but less marked. Five days later sudden rise of temperature. W. B. C., 13,000. Jaundice. April 12, collapse, death. Impression: At time of test moderate acute passive congestion of liver. Nothing to suggest any chronic changes. Blood examination: Hb., 86 per cent; R. B. C., 5,000,000. March 29, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 33 per cent. No autopsy. Thrombosis at site of injection.

(41.) No. 88884. E. G., age 42, male, colored.

Clinical diagnosis: Myocardinal insufficiency. Dilated aortic arch. Syphilis. Chronic nephritis. History of cardiac failure for only six months. On admission: Orthopnœa, anascarca, dilated heart, gallop rhythm, liver not made out an account of ascites. At time of test, firm liver edge palpable three fingers' breadth below costal margin. Same symptoms as on admission but less marked. Impression: Moderate recent chronic passive congestion of liver and no evidence of chronic lesion. Blood examination: Hb., 75 per cent; R. B. C.. 6,600,000. April 27, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 32 per cent. On May 7, liver no longer felt. Symptoms have cleared up.

(42.) J. J., age 44, male, colored.

Clinical diagnosis: Myocardial insufficiency, syphilis, aortic insufficiency. Very severe break in compensation. General edema; edema of lungs. Dilated heart. Very large tender liver. Several previous breaks. In extremis at time of both tests. April 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 5 per cent. May 19, 1913, injected 300 mg. Amount in urine, 0. Amount in stools, 24 per cent. *Autopsy:* 3946. Diag.—Syphilis, aortitis, aortic stenosis and insufficiency, myocarditis, chronic passive congestion of lungs and abdominal viscera, etc. Liver weighs 1220 gm. The microscopical section shows well marked passive congestion with central atrophy, obviously of long duration. The liver cells in many lobules have completely disappeared, leaving only a thickened reticulum. The lobulation is slightly irregular. Fatty degeneration appears in the middle zone. About one-half of the parenchyma cells nearly normal. Some of the lobules show the bile canaliculi distended with yellow, hyaline plugs. This condition is present in the central portion of the lobules, and absent in the periphery.

(43.) No. 89247. T. G., age 52, male, colored.

Clinical diagnosis: Arteriosclerosis, myocardial degeneration, chronic nephritis. Shortness of breath, palpitation, nycturia, transient swelling of feet and ankles for five months. Examination: Relative cardiac dulness 17 cm. to left, 5.5 to right, arrhythmia. No myocardial insufficiency. Liver dulness to one finger's breadth above costal margin; edge not felt. No evidence of liver disease. Blood examination: Hb., 72 per cent; R. B. C., 4,600,000. May 17, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 30 per cent. May 24, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 28-30 per cent. Thrombosis at site of injection.

(44.) No. 89149. I. W., age 48, male, colored.

Clinical diagnosis: Mitral insufficiency. Arteriosclerosis. Old mitral insufficiency. History of a break in 1910. Now for past two months, dyspnœa, cough, palpitation. Examination: R. C. D., 15 cm. to left in sixth interspace, loud systolic murmur, slight edema of lungs, none of ankles. Liver to costal margin; edge not definitely felt. Nothing to indicate liver injury. Blood examination: Hb., 78 per cent; R. B. C., 5,000,000. May 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 28 per cent.

(45.) No. 89301. I. B., age 26, male, white.

Clinical diagnosis: Miliary tuberculosis. Tuberculous pericarditis. Liver not below costal margin. Edge not felt. Slight irregular fever. Blood examination: Hb., 60 per cent; R. B. C., 5,200,000. May 27, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 21 per cent. *Autopsy:* 3941. Tuberculous pericarditis. Miliary tuberculosis of lungs, liver and spleen. Ascites, hydrothorax, chronic passive congestion of viscera, etc. Liver weight 1775 gm. The microscopical section shows well marked passive congestion with atrophy of the central portion of the lobule. About two-fifths of the parenchyma is involved in the central atrophy. There is little fatty degeneration. The margin of the parenchyma is normal.

(46.) No. 89417. S. R., age 33, female, colored.

Clinical diagnosis: Myocardial insufficiency, syphilis, aortic insufficiency. At time of first test marked broken compensation with anasarca. Edema of lungs. Liver hard, tender, down almost to umbilicus. At second test condition improved but still hard liver a palm's breadth below costal margin. Impression: Probably marked nutmeg liver. No signs of hepatic insufficiency. April 24, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 7 per cent. May 8, 1913, Amount in urine, 0. Amount in stools, 33 per cent.

(48.) J. M., age 45, male, colored.

Clinical diagnosis: Myocardial insufficiency, syphilis, aortic insufficiency. Several previous breaks. At time of test slight edema of ankles and lungs, dyspnœa, enlarged heart. Liver four fingers' breadth below costal margin and hard. Impression: Moderate induration of liver. No signs of hepatic insufficiency. June 19, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 20-22 per cent.

(49.) No. 88072. J. N., age 52, male, white.

Clinical diagnosis: Cirrhosis of liver. *First admission in* 1907: Long alcoholic history. At that time for 19 weeks dropsy, "misery in stomach," and constipation. Blood in stools. Tapped six times before admission, one "gallon" removed each time. Heart and lungs clear. Liver edge 2.5 cm. below ensiform. Edge uneven and firm. Ascites and edema of legs. Tapped 27 times between March, 1907, and October, 1907. Total of 272 L. removed. Discharged. Improved. Abdomen still slightly swollen and some edema of legs. No fever, leucocytosis, drowsiness, or any signs of hepatic toxæmia. Symptoms due apparently more to portal obstruction than to liver insufficiency. *Second admission, June, 1911.* Meanwhile tapped three to four times, but no other symptoms. Felt well. Returned on account of increase in size of abdomen and sharp pains below costal margin. Slight jaundice for one year. Heart and lungs clear. Liver edge 3 cm. below costal margin. Tenderness in gall bladder region, with rigidity of recti. Increased dulness in gall bladder region. Slight jaundice. Movable dulness in flanks. W. B. C., 14,000. Irregular fever up to 100° F. No note of tapping during this admission. Discharged July 5. Temperature normal, feeling well, no shifting dulness. Levulose test by Dr. Churchman. Positive on 100 gm. *Third admission.* Recalled to have test made. Good health since 1911. Complains only of occasional slight dyspnoea. Is able to work. Abdomen pendulous, walls soft and flabby. Relative hepatic dulness begins in fifth interspace and absolute hepatic dulness in sixth interspace. Dulness to 3 cm. below costal margin in right mamillary line. Edge felt firm but regular. No ascites. No dilated superficial veins. No edema of extremities. Heart and lungs clear. Urine clear. No fever. Impression: Undoubted cirrhosis of liver. Never any symptoms to suggest hepatic insufficiency except perhaps on second admission. Symptoms due to portal obstruction which at present is perfectly compensated. Blood examination: Hb., 75 per cent; R. B. C., 4,500,000. March 10, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 23 per cent. Fibrinogen content of blood by Dr. Whipple normal.

(50.) Bay View. D. M., age 56, male, white.

Clinical diagnosis: Cirrhosis of liver. Shortness of breath 20 years. Periodic edema of ankles. Markedly alcoholic. For three years, swelling of abdomen and epigastric pains. At time of test general bronzing. Patient is torpid and dull. Liver, 4 cm. below costal margin in right mamillary line. Superficial veins dilated. Caput medusæ. Edge of liver firm. Spleen palpable. Very slight ascites. March 26, 13, injected 400 mg. Amount in urine, 0. Amount in stools, 6 per cent. No autopsy.

(52.) Surg. No. 31832. K. F., age 48, female, white.

Clinical diagnosis: Carcinoma of stomach, metastases to liver. For two months chills, sweating, fever, pain in epigastrium, radiating to right shoulder, vomiting and loss of weight. Liver dulness from third interspace. Firm, smooth rounded liver edge palm's breadth below costal margin. Surface smooth. *Exploration:* Liver enormously enlarged, studded with large carcinomatous nodules. No symptoms of hepatic insufficiency. No glycosuria. March 19, 1913, inected 400 mg. Amount in urine 1.5 per cent. Amount in stools 8 per cent. *Autopsy:* 3903. Carcinoma of pylorus. Metastases to liver. Atrophy and focal necrosis of liver parenchyma with fatty degeneration. Practically entire liver tissue involved. The microscopic section shows numerous

tumor nodules. Medullary cancer. Liver lobules show central, fatty degeneration and scattered areas of hyaline necrosis. In some areas these areas of necrosis are very extensive; in other lobules absent. The liver parenchyma adjoining cancer growth shows extreme atrophy and fatty degeneration.

(53.) Bay View. A. H., age 67, female, white.

Clinical diagnosis: Carcinoma of gall bladder. Cholelithiasis. Six or seven attacks of jaundice in past 40 years. Two weeks before admission—weakness, loss of appetite, jaundice. Examination: Emaciation, moderate jaundice, large mass, firm and rounded, from right costal margin down to 4 cm. below level of umbilicus (gall bladder?). Liver edge well felt; smooth, normally firm. Wassermann reaction 100 per cent +. Blood examination: Hb., 55 per cent. April 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 18 to 20 per cent. Two and one-half months after onset jaundice more intense than ever.

(54.) No. 88424. E. S., age 33, male, white.

Clinical diagnosis: Cancer of stomach (metastases). For two months attacks of vomiting, nausea and pain following about two hours after eating. For past week vomitus bloody. Lost 80 pounds in three months. Examination: Emaciation; anemia; firm, nodular, transverse mass in epigastrium, thought by some to be liver, but finally was thought to be stomach or omentum. Liver dulness only to costal margin. Edge not felt. Blood examination: Hb., 38 per cent; R. B. C., 3,600,000. March 31, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 4 per cent. Stools unsatisfactory. April 8, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 22 per cent. Good specimens. *Exploration:* Carcinoma of stomach, metastases in glands and omentum. Liver smooth, no nodules made out (Finney). Death.

(56.) No. 87656. M. S., age 40 (?), female, colored.

Clinical diagnosis: Carcinoma of cervix uteri. One month before test general abdominal carcinomatosis found on abdominal exploration. At time of test some ascites; irregular nodular masses. Impression was that none of these were connected with liver, that dulness came to costal margin and that edge was not felt. Blood examination: Hb. 40 per cent; R. B. C., 4,300,000. March 5, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 39 per cent. Discharged April 10, 1913, without marked change in condition.

(57.) No. 89781. J. T., age 45, male, white.

Clinical diagnosis: Primary cancer of liver. For two months weakness, loss of weight, abdominal pain. *Exploratory laparotomy:* extensive inoperable primary cancer of liver. At time of test (post operative) great weakness and emaciation. Hb., 88 per cent. June 20, 1913 injected 400 mg. Amount in urine, trace. Amount in stools, 6 to 7 per cent.

(58.) No. 87983. M. B., age 27, male, white.

Clinical diagnosis: Suspected amebic abscess of liver. Ill about two months. Septic fever up to 102° F. W. B. C., 12,000. Liver dulness from fifth interspace to 6 cm. below costal margin in right mammillary line. Edge blunt, round. Surface smooth and tender. Careful *exploration* March 19 by Dr. Hulsted "was negative, with the exception of the enlargement of the right lobe of the liver, and the finding of a few adhesions between the right lobe and the dome of the liver." The peritoneum at "the lower portion of the right lobe which presented in wound was very soft and almost fluctuant." Blood examination: Hb. 75 per cent; R. B. C., 4,000,000. March 10, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 33 per cent. Death. No autopsy.

(60.) No. 88925. F. R., age 40, male, colored.

Clinical diagnosis: Hepatitis, periostitis, syphilis, tuberculosis (?) tuberculous peritonitis (?) and perihepatitis (?). Ascites of obscure origin, large hard spleen. No evidences of collateral circulation. Liver just felt at costal margin, indefinite, not hard. Impossible to tell extent of anatomical liver change. No clinical signs of hepatic insufficiency. Blood examination: Hb., 40 per cent; R. B. C., 2,600,000. April 27, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 30 per cent. Case not followed after leaving hospital.

(61.) No. 88861. E. P., age 19, male, colored.

Clinical diagnosis: Tuberculosis (eye test). Splenomegaly. Liver easily felt one finger's breadth below costal margin; edge smooth and firm. Spleen easily felt; edge firm and sharp. Diagnosis not clear; chronic malarial infection suspected. No parasites found. No clinical signs of hepatic insufficiency. Blood examination: Hb., 90 per cent; R. B. C., 5,000,000. May 10, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 23 per cent.

(62.) No. 89082. J. McC., age 52, male, white.

Clinical diagnosis: Tuberculous peritonitis? Hepatitis? Syphilis (w). Mitral insufficiency. Arteriosclerosis. Symptoms: For about a month weakness, shortness of breath, abdominal pain, swelling of feet. On admission, mitral murmur, heart 11 cm. to left, liver one finger's breadth below costal margin; edge not felt, edema of legs, ascites. Diagnosis not clear; most likely cause of findings is myocardial failure, perhaps with chronic peritonitis. No symptoms of hepatic insufficiency. Blood examination: Hb., 60 per cent; R. B. C., 4,400,000. May 17, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 31 per cent. June 10, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 31 per cent.

(63.) No. 89439. J. K., age 56, male, colored.

Clinical diagnosis: Acute cholecystitis. Previously well. Acute onset with nausea, pain below right costal margin. Rigidity of upper right rectus, slight icterus. Leucocytosis, 22,000. Rapid recovery. Blood examination: Hb., 70 per cent; R. B. C., 4,000,000. May 29, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 30 per cent.

(64.) No. 89593. H. A., age 52, male, white.

Clinical diagnosis: Tuberculous peritonitis (?). Cirrhosis of liver (?). For six months increasing weakness and abdominal pain, with gradual ascites. Hemorrhoids 8 years. Emaciation, anæmia, ascites, paracentesis—cloudy, brownish fluid, specific gravity 1017. Liver felt almost at umbilicus; surface irregular, firm, and slightly tender. June 3, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 22 per cent.

(65.) Bay View. E. T., age 33, male, colored.

Clinical diagnosis: Syphilis of liver, syphilitic endarteritis, mitral insufficiency. Very large liver reaching to level of umbilicus in right flank, slightly irregular, and knobbed. Slightly tender. No other masses. Ascites. April 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 18 to 22 per cent.

(66.) Bay View. G. A. S., age 27, male, colored.

Clinical diagnosis: Syphilitic hepatitis? Amyloid? Pulmonary tuberculosis. Definite primary and tertiary lesions with positive Wassermann reaction. Two months before admission: swelling of legs, shortness of breath, cough and expectoration. Examination: Ascites, liver very large, smooth, not tender, does not pulsate, 8 cm. below costal margin. Moderate, irregular fever up to 101° F. in afternoon. Hb., 75 per cent. April 22, 1913, injected 400 mg. Amount in urine, 0. Amount in stools, 40 per cent. Autopsy: B. V. 41. Liver.—Microscopical section shows well-marked passive congestion, with central atrophy involving about ⅓ of each lobule, where the liver cells have undergone advanced atrophy, and in some instances even complete death. The sinusoids are engorged with blood. The liver lobulation is quite regular. Kidneys show advanced chronic nephritis. Lungs.—Bronchiectasis and organizing bronchopneumonia.

TESTS FOR HEPATIC FUNCTION AND DISEASE UNDER EXPERIMENTAL CONDITIONS.

PHENOLTETRACHLORPHTHALEIN.

By G. H. WHIPPLE, M. D., T. C. PEIGHTAL AND A. H. CLARK.

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In an earlier paper (Whipple, Mason and Peightal)[1] we have called attention briefly to three tests which may be of value in determining liver injury and estimating liver functional capacity. The estimation of blood plasma or serum *lipase* will determine the presence of acute liver injury when this ferment lipase increases greatly (five or eight times normal). It does not, however, give an accurate idea of the *amount* of acute liver injury, although a grave injury will show a higher lipase reading than a moderate injury. The test is of value as a qualitative rather than as a quantitative test and is of importance in suspected eclampsia, chloroform poisoning, yellow atrophy, cholangitis, etc.

Fibrinogen is a second factor of importance, and when this element of the blood plasma is very low it gives certain evidence of a grave hepatic insufficiency; but it may not be low in all types of liver cirrhosis. A rough estimate of the fibrinogen content in the blood can be made very easily by clotting a little plasma with calcium and testing the toughness of the clot with a glass rod, if possible with a normal control and fractional dilutions. With practice it is very easy to determine whether the fibrinogen is greatly diminished in amount, when quantitative estimates can be made if desirable.

Phenoltetrachlorphthalein has been studied especially by Abel and Rowntree[2] who have determined its physiological and therapeutic properties. Through their kindness we have been able to use this drug in our work with various experimental liver conditions. The experimental work detailed below will show that this drug, when given intravenously and collected in the feces and urine, can give important evidence concerning the amount of liver injury and the degree of functional impairment.

It will be found that after an acute liver injury there is an immediate drop in liver phthalein output in the feces and this drop corresponds very closely to the degree of injury, falling to zero with a fatal intoxication. Further, this phenoltetrachlorphthalein, which does not come through in the urine of a normal dog, will appear very promptly in the urine after the liver injury is established. It seems probable that the phthalein is so modified by contact with the injured liver cell that it can pass the kidney filter which is impermeable to the unchanged phthalein. As the injury to the liver is repaired the curve of phthalein output in the feces will rise to normal and the urine output sinks to zero.

METHOD.

The various methods for producing liver injury are described under the various experiments. The routine followed in giving the phthalein may be outlined as follows: As a rule the experiments were done in groups, three to six animals being injected at the same time, at least one normal dog serving as control. When a dog weighs less than 20 pounds, 0.1 gm. phthalein is given as a rule, and 0.2 gm. of the drug when over 20 pounds. Subcutaneous injection does not give uniform results. Practically all these injections were given by means of a needle introduced into the jugular vein, and with care this procedure can be repeated very frequently without causing thrombosis. Laceration of the vein may predispose to a localised thrombus involving perhaps only one or two inches of the vein. Injection is usually done about 11 a. m. or 5 p. m. and followed by about 200 cc. of water by stomach tube to promote diuresis. Urine is collected usually by a catheter or from a clean metabolism cage at 5 p. m. or the following morning and a purge given, usually magnesium sulphate or croton oil. Feces are collected at the end of 20-24 hours and the feces subsequently passed are collected to insure complete phthalein collection. With brisk purgation the second fraction will rarely contain more than a trace of phthalein. When purgation is not brought about because of vomiting or other factors the feces on the second or third day may contain the usual amount of phthalein, but there may be some loss, perhaps from absorption or destruction. It is not safe to draw deductions from a slightly lowered output under these circumstances.

The method of extracting the phthalein from the feces has been modified from time to time as the work progressed and the following method in our hands gives the most uniform results working with dog feces. The determinations were done as a rule in triplicate, each worker using a separate fraction of the feces emulsion and making independent readings. When the readings were not uniform, repeated determinations were done, but it was unusual for the readings to vary more than 5 per cent. The feces must be collected carefully from metabolism cages, the residue being washed out with a wash bottle and spatula to remove the last traces. The total feces are diluted to one or two litres as may be necessary in the collection and washing. The mixture is made alkaline with 10 cc. sodium hydrate (40 per cent) and shaken until a uniform mixture is obtained. If there is any doubt of this a second determination the following day is advisable when solution is complete. One-tenth of this mixture is taken and diluted to 500 cc. with water and 3-4 cc. sodium hydrate. After shaking and sedimentation over night this second solution may be perfectly clear, and with filtration and dilution can be read directly in the colorimeter. When no precipitation is needful we believe the readings to be most accurate, and have used such solutions to control the methods of precipitation which at times remove some of the phthalein. Under such circumstances the basic lead acetate precipitation may drop the reading in parallel observations as much as 5-10 per cent and we must assume that the lead acetate may hold some of the phthalein, even in alkaline solution.

The second fraction, as a rule, must be cleared before a suitable reading can be made. Of the 500 cc. solution 100 cc. are taken for precipitation with 4-5 cc. of a calcium solution (calcium chloride 90 gm., ammonium hydrate (conc.) 10 cc. and water to 500cc.). This throws down an abundant precipitate on shaking and the mixture is made up to 200 cc. with 3 to 4 cc. sodium hydrate and water. After standing and filtration a perfectly clear solution is obtained which can be read directly against a standard

solution of phthalein, 10 mg. to 1 litre. In some feces, normal or otherwise, this extraction will give a dirty, reddish color, which is difficult to read in the colorimeter, but varying the amounts of the calcium reagent and sodium hydrate will usually give a clear lavender red tint which can be estimated accurately. At times a solution may be cleared up after filtration by adding a little more sodium hydrate and standing several hours.

The estimation of phthalein in the urine is very simple:—addition of a little hydrate and calcium solution with dilution to a proper degree for reading. The normal dog often excretes traces of phthalein in the urine sufficient to give a rose red with alkali, but not sufficient for a reading. When phthalein to the extent of 0.2 per cent or more is excreted in the urine, we believe it indicates liver injury. Bile pigments give difficulty, but the addition of calcium with removal of the precipitated bile pigments will give a solution suitable for the colorimeter.

EXPERIMENTAL OBSERVATIONS.

Fatal Chloroform Poisoning and Liver Necrosis.

Dog 12-50.—Fox terrier, female; weight 17 lbs.

April 1. 1 p. m., *phthalein 0.1 gm.* intravenously. Weight 17¾ lbs. 4 p. m., urine contains no phthalein.

April 2. Feces abundant. *Phthalein 35-40 per cent.*

April 12. Dog in excellent condition; weight 18 lbs. 12 m., *phthalein 0.1 gm.* intravenously.

April 13. Abundant feces. *Phthalein 45 per cent.*

April 15. 1 p. m., *phthalein 0.2* gm. intravenously. Weight 19¼ lbs. 4 p. m., urine contains traces of phthalein.

April 16. Abundant fluid feces. *Phthalein 40 per cent.*

May 20. Dog is in a good condition, weight 18½ lbs. *Chloroform anæsthesia for three hours.*

May 21. 11 a. m., *phthalein 0.1 gm.* intravenously. Dog appears rather sick and intoxicated. 5 p. m., *urine* by catheter contains abundant *phthalein, 8 per cent.*

May 22. 11 a. m., abundant feces and vomitus contains only faint traces of phthalein. Dog shows definite icterus of the skin and mucous membranes and appears very sick, dull and drowsy. Weight 15¾ lbs. Urine by catheter contains traces of phthalein. 11.30 a. m., *phthalein 0.2 gm.* intravenously. 1.30 p. m., death. Abundant feces were passed shortly before death. *Phthalein 9.* Autopsy done at once. Thorax and heart negative. Lungs show a few small purple patches of bronchopneumonia or atelectasis. Liver shows extensive central necrosis involving all the lobules. Microscopical section: The greater part of the liver has undergone complete hyaline necrosis, most of the injured liver cell nuclei having disappeared. Only a few rows of intact liver cells remain about the portal spaces and many of these cells show advanced fatty degeneration. Wandering cells of all descriptions are numerous in the areas of necrosis. The bile ducts are normal, but the larger ones in places are distended with colloid like material. The necrosis involves more than four-fifths of the liver parenchyma of each lobule. This is a typical example of fatal delayed chloroform poisoning. In places there are subserous hæmorrhages, particularly in the pelvis and about the bladder.

Intestinal contents removed carefully. Phthalein present only in traces. Gall bladder contains only the faintest trace of phthalein.

Table I. Dog 12-50.—Chloroform Poisoning.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 1	.100	40	0	....	17¾	Normal.
12	.100	45	..	....	18	Do.
15	.200	40	Traces.	....	19¼	Do.
May 20	....	..	..	Chlorof'm 3 hrs.	...	
21	.100	Traces.	8	....	18½	Icterus marked.
22	.200	do.	(?)	....	15¼	
	Death	Traces in bile.	..	....	...	Liver lobules ⅘ necrosis.

Chloroform Poisoning, Severe and Mild.

Dog 12-48.—Small fox terrier, female; weight 14 lbs.

April 3. 12 m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains no phthalein.

April 4. Abundant feces. *Phthalein 47 per cent.* Urine contains no phthalein.

April 8. 11 a. m., *chloroform anæsthesia for 1¾ hours.* At the end of the anæsthesia intravenous injection of *phthalein 0.1 gm.* 5 p. m., vomitus abundant. *Phthalein 12 per cent.*

April 9. No feces. Urine soiled with some vomitus and contains phthalein 0.1 per cent. Dog appears quite sick.

April 10. 10 a. m., night urine contains traces of phthalein. One fluid stool. *Phthalein 7-9 per cent.* 12 m., urine contains no phthalein, but abundant bile pigment. 3 p. m., *phthalein 0.1 gm.* intravenously; weight 11½ lbs.

Plasma Lipase .10- .10 Initial alkalinity.
1.10-1.30 Butyric acidity.

1.20-1.40 Acid production.

Plasma clots are decidedly soft and fibrinogen estimated at about 100 mgs., which is about 25 per cent of normal. 5 p. m., urine contains abundant phthalein 1½ per cent. 7 p. m., urine contains phthalein 1 per cent. 9 p. m., urine plus vomitus contains phthalein 1½ per cent.

April 11. 9 a. m., vomitus and urine of night, phthalein 2 per cent. 3 p. m., urine contains faint traces of phthalein. No feces. 4 p. m., two-third fluid stools following croton oil. Phthalein 25 per cent.

April 12. Dog is improving rapidly and eats well.

April 15. 1 p. m., *phthalein 0.1 gm.* intravenously; weight 12½ lbs. 4 p. m., urine contains phthalein 0.1 per cent.

April 16. 10 a. m., abundant fluid feces. *Phthalein 45 per cent.*

May 28. Dog is in excellent condition and weighs 12 lbs. 5 p. m., *Phthalein 0.1 gm.* intravenously.

May 29. Urine contains no phthalein. Feces contain *40-44 per cent.*

May 30. Abundant fluid feces contain no phthalein.

June 2. 3 p. m., *chloroform anæsthesia 1 hour.*

June 3. Dog seems normal; weight 11 lbs. 11 a. m., *phthalein 0.1 gm.* intravenously.

Plasma Lipase .10-.10 Initial alkalinity.
.60-.50 Butyric acidity.

.70-.60 Acid production.

5 p. m., urine contains phthalein 1½ per cent. but no bile pigments.

June 4. No feces. Dog is normal.

June 5. Abundant feces. Phthalein 43 per cent.

Table II. Dog 12-48.—Chloroform Poisoning.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 3	.100	47	0	....	14	Normal.
8	.100	20	..	Chloroform 1¾ hrs.	11¾	Phthalein given at end of anæsthesia.
10	.100	25	4	....	11½	Lipase 5 times normal.
16	.100	45	0.1	....	12½	
May 28	.100	44	0	....	12	Dog normal.
June 2	....	..	..	Chlorof'm 1 hr.	..	
3	.100	43	1.5	....	11	Lipase 3 times normal.

The two preceding experiments (Dogs 12-50 and 12-49) show the prompt drop in phthalein output in the feces. In the first experiment there is a drop from 40 per cent excretion to a mere trace in a fatal chloroform poisoning, and in the second a prompt drop from 47 per cent to 20 per cent and 25 per cent with recovery to 45 per cent following repair of the liver injury within one week. The urine phthalein rose from zero, which is normal, to 8 per cent in the fatal case and to 4 per cent in the case with recovery. In the second experiment a slight degree of liver injury produced by one hour chloroform anæsthesia caused no drop in the phthalein of the feces, but an output of 1½ per cent in the urine and a rise in plasma lipase. From the last two observations we may conclude that this short chloroform anæsthesia caused actual liver injury, but did not upset the functional capacity of the liver. This corresponds with the clinical observations of this animal.

The following experiment (Dog 12-78) shows the same prompt drop in phthalein output in the feces due to a chloroform anæsthesia of two hours. The drop was from 53 per cent to 30 per cent and finally to 20 per cent, with complete recovery in two weeks to 46 per cent. The dog was then poisoned fatally with phosphorous and died on the fourth day after the last dose. The phthalein output in feces fell to zero on the day before death, but the urine output rose from zero to 4⅛ per cent. The curve of phthalein excretion in the feces shows in a striking manner (Chart I).

CHLOROFORM AND PHOSPHOROUS POISONING.

Dog 12-78.—Large bull-dog, female; weight 38½ lbs.

April 1. 1 p. m., *phthalein 0.2 gm.* intravenously. 5 p. m., urine by catheter contains traces of phthalein.

April 2. Abundant feces. *Phthalein 50-53 per cent.*

April 21. 3 p. m., *chloroform anæsthesia 1 hour 50 minutes.*

April 22. Dog seems practically normal; weight 39 lbs. 12 m., *phthalein 0.2 gm.* intravenously. Blood plasma appears normal except for slight icteric tint. Clots in 7 minutes with the formation of a firm clot.

Plasma Lipase .10- .10 Initial alkalinity.
1.10-1.35 Butyric acidity.
1.20-1.45 Acid production.

5 p. m., urine by catheter contains bile. No albumin. Phthalein 2½ per cent.

April 23. Abundant feces. *Phthalein 30 per cent.* Dog has vomited a few times and this is the only sign of intoxication.

April 25. 11 a. m., dog is active and normal. *Phthalein 0.2 gm.* intravenously. 4 p. m., urine contains phthalein 1 per cent.

April 26. Abundant feces. *Phthalein 19-20 per cent.*

May 9. Dog in excellent condition; weight 37 lbs. 12 m., *phthalein 0.2 gm.* intravenously.

May 10. 10 a. m., one solid stool. Phthalein 23 per cent.

May 11. 1 soft stool. *Phthalein 20-26 per cent.* 1 p. m., *phosphorous 10 mgs.* in oil given subcutaneously.

May 12. Dog looks rather sick.

May 14. Dog has vomited food. Icterus is not present.

May 15. Dog appears better. *Phosphorous 10 mgs.* in oil given subcutaneously.

May 16. Dog appears pretty well.

May 18. Dog is quite sick, refuses food and vomits. Slight icterus noted in the scleræ. 1 p. m., *phthalein 0.2 gm.* intravenously.

May 19. 9 a. m., dog found dead, but quite warm. Urine beneath cage (150 cc.) very concentrated and contains large amounts of bile pigments. Phthalein 4 per cent. Urine in bladder at autopsy 20 cc. Phthalein ½ per cent. Autopsy shows slight pleural effusion and bilateral hæmorrhage pneumonia and œdema. Oedema well marked in mediastinal tissues. Entire contents of the intestinal tract collected and washed from intestinal mucosa with care. *Phthalein* present in *merest trace.* Bile and gall bladder contain traces of phthalein perhaps a fraction of 1 per cent.

Liver in gross shows conspicuous lobulation and evidence of fatty change. Microscopical section: All the liver cells show fatty degeneration and contain medium and small-sized fat droplets. Necrotic liver cells scattered everywhere throughout the lobule are numerous. The portal tissues are rather œdematous and filled with wandering cells, some of which contain a yellow granular pigment. The capillaries between the strands of liver cells are engorged with blood cells and phagocytes; wandering cells of every description are numerous. Occasional mitotic figures are seen in liver cells. The bile canaliculi are not conspicuous. This is an excellent example of a subacute type of phosphorous injury to the liver. The irritant type of hæmorrhagic bronchopneumonia associated with œdema is quite common in dogs given an overdose of phosphorous.

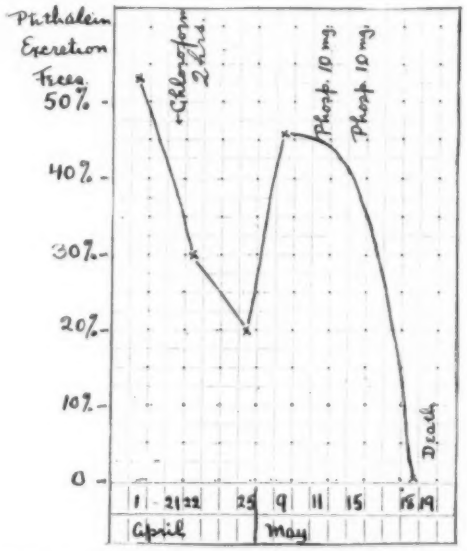

CHART I. DOG 12-78.—Chloroform and Phosphorous Poisoning.

TABLE III. DOG 12-78.—CHLOROFORM AND PHOSPHOROUS POISONING.

Date.	Phthalein given.	Phthalein excreted. Feces.	Phthalein excreted. Urine.	Liver injury.	Weight.	Remarks.
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 1	.200	53	+	....	38½	Normal.
21	....	..	..	Chlorof'm 2 hrs.	...	
22	.200	39	2½	....	39	Lipase 5 times normal.
25	.200	20	1	....	39½	Recovery rapid.
May 9	.200	..	..	....	37	
11	....	46	..	Phosp. 10 mg.	...	Purgation delayed.
15	....	..	..	Phosp. 10 mg.	...	
18	....	..	..	Icterus.	...	Dog vomits constantly.
18	.200	..	4½	....	...	
19	....	Intestinal traces.	..	....	...	Death—bile phthalein, ¼ ± %.

Severe Phosphorous Poisoning.

Dog 12-53.—Adult male mongrel; weight 16¼ lbs.

April 1. 1 p. m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine by catheter shows very faint trace of phthalein.

April 2. Feces abundant. *Phthalein 45 per cent.*

May 13. 3 p. m., *phthalein 0.1 gm.* intravenously. 8 p. m., urine contains no phthalein.

May 14. Abundant feces. *Phthalein 47 per cent.* Fowler's solution 2½ cc. given by stomach tube.

May 15. Dog has vomited and acts rather sick.

May 16. Given ¼ grain morphia followed in two hours by 3 cc. of Fowler's solution by stomach. Weight 18 lbs.

May 23. 5 p. m., dog does not appear poisoned; weight 18 lbs. *Phosphorous 5 mgs.* given in oil subcutaneously.

May 28. Dog seems pretty well; weight 18 lbs. 5 p. m., *phthalein 0.1 gm.* intravenously.

May 29. Urine of previous night contains no phthalein, which speaks against any definite injury by the dose of phosphorous given five days previously. Abundant feces collected in the afternoon. *Phthalein 45-50 per cent.*

May 30. 4 p. m., *phosphorous 7.5 mgs.* given subcutaneously. Weight 16 lbs. Feces passed during the night abundant. *Phthalein 2-3 per cent.*

June 2. Dog looks pretty well, weight 17 lbs. Icterus noted for the first time in scleræ and skin, indicating fatal poisoning as a rule. 11 a. m., *phthalein 0.1 gm.* intravenously. 3 p. m., urine contains much phthalein. 4½ per cent. Dog vomits repeatedly but the vomitus contains no bile, being made up chiefly of clear fluid and mucus. 5 p. m., urine contains abundant phthalein, 1½ per cent. Bile is very abundant.

June 3. 10 a. m., urine and vomitus below cage contain traces of phthalein. 10 a.m., abundant fluid feces. *Phthalein* present in only the *faintest traces*, impossible to read in ten times the usual concentration. Jaundice of skin is quite deep. 12 m., urine contains abundant bile. Phthalein 0.1 per cent ±.

June 4. 10 a. m., soft feces contain mere traces of phthalein. General condition is unchanged. Dog is steadily losing weight; weight 15 lbs. 12 m., *phthalein 0.2 gm.* intravenously. 3 p. m., urine contains abundant phthalein, 3 per cent. 4 p. m., urine contains phthalein, 3½ per cent. Night urine contains phthalein, 2-4 per cent. Bile is constantly present.

June 5. 9 a. m., fresh urine contains phthalein, 0.7 per cent. Dog does not vomit and drinks considerable water; weight 15 lbs. 2 p. m., abundant fresh urine contains much bile and phthalein, 1 per cent.

June 6. 11 a. m., condition is about the same; weight 14½ lbs. Night urine contains a good deal of phthalein. 12 m. 500 cc. of normal saline subcutaneously. 3 p. m., soft soapy looking feces passed and contain small amounts of *phthalein. 1¼ per cent.* Urine contains traces of phthalein. Jaundice remains intense, but dog drinks milk and does not vomit.

June 7. 10 a. m., clean urine contains definite traces of phthalein and much bile. Dog is improving; weight 15½ lbs. 12 m., urine is rich in bile and contains only traces of phthalein.

June 8. 10 a. m., urine contains much bile and faint traces of phthalein. Feces are abundant, gray and putty like. Phthalein 0; not the faintest trace. Icterus is becoming less intense.

June 9. 10 a. m., urine of night contains no phthalein; gives only faint test for bile pigments. Dog is quite lively and eats well; weight 16 lbs. 12.30 p. m., *phthalein 0.2 gm.* intravenously. 4 p. m., urine contains phthalein, 1½ per cent. 5 p. m., urine contains phthalein, 0.6 per cent.

June 10. 10 a. m., urine of night contains only faint traces of phthalein. Abundant feces collected in afternoon. Phthalein 33 per cent. Jaundice is clearing completely in skin and only faint traces in the scleræ. 4 p. m., *phthalein 0.1 gm.* intravenously.

June 11. 10 a. m., urine of night contains phthalein, 1 per cent. 3 p. m., abundant feces. *Phthalein 22 per cent.* Dog seems almost normal, and the urine is quite free from bile.

June 16. Appearance normal; weight 16¾ lbs. Urine is free from pigment.

July 10. 10 a. m., dog appears well; weight 15¾ lbs. *Phthalein 0.1 gm.* intravenously. 5 p. m., urine, *phthalein 0.3 ± per cent.*

July 11. Abundant feces. Phthalein 60 per cent (Hypersecretion ?).

July 14. Dog as usual; weight 16 lbs. Sacrificed. Autopsy at once. All the organs are normal in gross. The bile papilla and bile ducts are normal. The gall bladder shows thickening and slight ulceration in its distal portion, with adherent slimy green material on the mucous surface. There are adhesions between the liver and omentum at this place. Liver on section

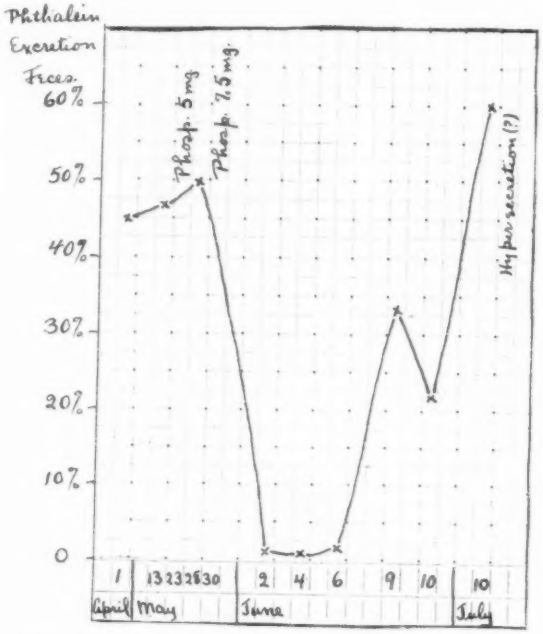

Chart II. Dog 12-53.—Phosphorous Poisoning and Repair.

Table IV. Dog 12-53.—Phosphorous Poisoning.

Date.	Phthalein given.	Phthalein excreted. Feces.	Phthalein excreted. Urine.	Liver injury.	Weight.	Remarks.
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 1	.100	45	0	....	16¼	Normal.
May 13	.100	47	0	....	18	Do.
14	....	..	..	Fowler's Sol. 2½ cc.	...	
16	....	..	..	Fowler's Sol. 3 cc.	18	
23	....	..	..	Phosp. mg.	18	
28	.100	50	0	....	18	
30	....	..	..	Phosp. 7½ mg.	16	
June 2	.100	..	6	....	17	Icterus.
3	....	Traces.	+	....	...	
[1]4	....	Traces.	+	....	15	Bile + + + in urine.
[2]4	.200	0	9½	....	15	Vomiting and prostration, dull and drowsy.
5	....	0	1.7	....	...	
6	....	¼	+	....	14½	Given 500 cc. salt infusion.
7	....	0	+	....	15½	
8	....	0	Traces.	....	...	Bile + + in urine.
9	....	0	0	....	16	Bile traces in urine.
9	.200	33	2	....	...	Dog eats and is lively.
10	.100	22	1±	....	...	Dog improving rapidly.
July 10	.100	60	0.3±	....	15¾	Hypersecretion (?).

[1] A. M. [2] P. M.

seems normal. Microscopical section: Normal liver parenchyma except for a slight increase in phagocytes about the portal areas. Some of these phagocytes contain yellow pigment. The repair is almost perfect.

The preceding experiment (Dog 12-53) illustrates the gravest type of phosphorous liver injury. The normal phthalein output was constantly 45-50 per cent, and after the phosphorous injury dropped to zero with a corresponding rise in the urine from zero to 6 or 9½ per cent. This is the only case in a considerable number of experiments where a dog recovered from phosphorous poisoning after a definite icterus had developed. The liver repair was considerably delayed in this instance, but finally after weeks the phthalein curve returned to normal and even showed evidence of hypersecretion (Chart II). Autopsy showed practically prefect repair of the liver injury.

PHOSPHOROUS POISONING—FATAL 6 DAYS.

Dog 12-88.—Fox terrier, male; weight 14¾ lbs.

April 3. 1 p. m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains no phthalein.

April 4. Abundant feces. Phthalein 43-47 per cent.

April 7. *Phosphorous 10 mgs.* in oil given subcutaneously; weight 14 lbs.

April 8. Dog shows no signs of intoxication. 12 m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine by catheter contains traces of phthalein.

Plasma Lipase .10- .10 Initial alkalinity.
1.45-1.10 Butyric acidity.

1.55-1.20 Acid production.

April 9. Abundant feces. *Phthalein 25 per cent.* Dog is pretty sick and refuses food.

April 10. 3 p. m., *phthalein 0.99 gm.* intravenously. Blood plasma clots normally but the blood clot is decidedly flabby indicating a drop in fibrinogen.

Plasma Lipase .10-.10 Initial alkalinity.
.60-.50 Butyric acidity.

.70-.60 Acid production.

6 p. m., urine contains abundant phthalein, 1½ per cent. 9 p. m., urine contains phthalein, 2½ per cent.

April 11. Abundant feces. *Phthalein* shows the *merest trace.* Dog is sick and keeps very quiet.

April 12. Dog shows definite jaundice of skin and scleræ. 12 m., *phthalein 0.1 gm;* weight 12½ lbs. 3 p. m., urine contains abundant bile. Phthalein 1 per cent.

April 13. Dog is very sick and has much bloody diarrhœa. No vomiting. Feces contain mere *traces of phthalein.* 3 p. m., dog very sick and was sacrificed. Autopsy performed at once. Blood clots formed soft jelly like masses indicating very little fibrinogen.

Plasma Lipase .10-.10 Initial alkalinity.
.65-.60 Butyric acidity.

.75-.70 Acid production.

Thorax, heart and lungs are normal. Spleen shows a few hæmorrhages beneath the capsule. Stomach contains black, tarry material. Duodenum is normal. Bile can be squeezed with ease through the bile papilla. The gall bladder is small and flabby. The bile in the gall bladder contains phthalein 1 per cent ±. Urine in the bladder contains abundant bile pigments and phthalein 2 per cent ±. There is a definite cloud of albumin. Kidneys show some linear abscesses, which are most numerous in the right kidney. Liver is pale and yellowish, obviously very fatty, quite soft and slightly smaller than normal.

Microscopical sections are negative except the liver. This shows advanced fatty degeneration, all the liver cells showing great numbers of fat droplets. The majority of them show a mere honeycombed protoplasm. Actual hyaline necrosis is rarely observed, but is present. Wandering cells are pretty numerous. The portal structures are normal.

TABLE V. DOG 12-88.—PHOSPHOROUS POISONING.

Date.	Phthalein given.	Phthalein excreted. Feces.	Phthalein excreted. Urine.	Liver injury.	Weight.	Remarks.
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 3	.100	45	0	....	14¼	Normal.
7	....	..	..	Phosp. 10 mg.	...	
8	.100	..	Trace.	....	13¾	Dog appears well.
9	....	25	.	....	...	Dog will not eat.
10	.090	..	4	....	14¼	
11	....	Traces.	..	....	...	Dog is sick.
12	.100	..	1 ±	....	12½	Icterus.
13	....	Traces.	2	....	...	
	Sacrificed.	Bile 1±	2±	....	...	Fibrinogen very low.

The preceding experiment (Dog 12-88) shows a typical example of phosphorous poisoning and extreme liver injury. It will be seen that the phthalein output in the feces fell rather slowly from the normal 45 per cent to 25 per cent on the second day, but practically to zero on the fourth day, and remained here until death on the sixth day. It is interesting to note that the lipase readings were highest on the day following the injection of phosphorous and fell somewhat as the intoxication progressed. We have observed the same lipase curve in fatal chloroform poisoning.

CHRONIC PHOSPHOROUS POISONING.

Dog 12-105.—White bull dog, female; weight 24¾ lbs.

April 18. 2 p. m., *phthalein 0.1 gm.* intravenously. 5.30 p. m., urine by catheter contains no phthalein.

April 19. Abundant feces. *Phthalein 32 per cent.*

April 22. 12 m., *phthalein 0.2 gm.* intravenously; weight 22½ lbs. 5 p. m., urine contains no phthalein.

April 23. Abundant feces. *Phthalein 39 per cent.*

May 23. Dog in good condition, weight 24 lbs. *Phosphorous 7½ mgs.* in oil given subcutaneously.

May 25. Dog looks somewhat intoxicated.

May 26. Dog appears sick but has no icterus; weight 22 lbs. 11 a. m., *phthalein 0.2 gm.* intravenously. 5 p. m., urine by catheter contains no bile. Phthalein 1.2 per cent.

May 27. Abundant feces. *Phthalein 22 per cent.*

May 28. Dog has diarrhœa and vomiting. Urine by catheter contains no albumin and no bile pigment.

May 30. 5. p. m., *phthalein 0.2 gm.* intravenously; weight 21½ lbs.

May 31. Feces abundant. *Phthalein 60 per cent.*

June 2. Dog has some diarrhœa; weight 21 lbs. *Phosphorous 5 mgs.* in oil subcutaneously.

June 10. Dog is in fair condition; weight 22 lbs. Phosphorous 5 mgs. in oil given subcutaneously.

June 18. Dog continues to have diarrhœa; weight 21½ lbs. 12 m., *phthalein 0.2 gm.* intravenously. 3 p. m., urine by catheter contains only faint traces of phthalein. Bile and albumin are absent.

June 19. Abundant feces. *Phthalein ½ per cent.*

June 23. Dog in fair condition; weight 20½ lbs. *Phosphorous 5 mgs.* in oil subcutaneously.

June 28. *Phosphorous 5 mgs.* in oil subcutaneously.

July 8. *Phosphorous 5 mgs;* weight 19¼ lbs.

July 10. Condition fair; weight 19½ lbs. 10 a. m., *phthalein 0.2 gm.* intravenously. 2.30 p. m., urine phthalein ¼ per cent. 3 p. m., phthalein 0.

July 11. Abundant feces. *Phthalein ½ per cent.*

July 24. Condition unchanged; weight 19 lbs. Sacrificed. Autopsy at once. All the organs are normal. Liver appears quite normal in gross. The bile passages show no abnormality. Microscopical section of liver is normal.

TABLE VI. DOG 12-105.—CHRONIC PHOSPHOROUS POISONING.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent	*Per cent.*		*Pounds.*	
Apr. 18	.100	32	0	....	24½	Normal.
22	.200	39	0	....	22½	Do.
May 23	....	..	..	Phosp. 7½ mg.	24	
26	.200	22	¼	....	22	Dog is sick.
30	.200	49	(?)	....	21½	Hypersecretion (?).
June 2	....	..	..	Phosp. 5 mg.	21	
10	....	..	..	Phosp. 5 mg.	22	Diarrhœa.
18	.200	42	Traces.	....	21½	Bloody diarrhœa.
23	....	..	..	Phosp. 5 mg.	20½	
28	....	..	..	Phosp. 5 mg.	...	
July 8	....	..	..	Phosp. 5 mg.	19½	
10	.200	44	¼	....	19½	

HYDRAZINE AND PHOSPHOROUS POISONING—EARLY CIRRHOSIS.

DOG 12-82.—Mongrel male; weight 17½ lbs.

April 5. 1 p. m., *phthalein 0.1 gm.* intravenously. *Hydrazine sulphate 0.1 gm.* subcutaneously. 7 p. m., urine by catheter contains abundant phthalein.

April 6. Feces abundant. *Phthalein 20-22 per cent.*

April 7. *0.2 gm. hydrazine* subcutaneously.

April 8. Dog eats well; weight 16¼ lbs.

Plasma Lipase .10-.10 Initial alkalinity.
.10-.10 Butyric acidity.

.20-.20 Acid production.

12 m., *phthalein 0.1 gm.* given intravenously. 5 p. m., urine contains phthalein, turning deep red with hydroxide.

April 9. Abundant feces. *Phthalein 25-27 per cent.* Dog does not appear intoxicated.

April 10. 3 p. m., weight 16½ lbs. *Phthalein 0.1 gm.* intravenously. 6 p. m., urine contains phthalein, 1½ per cent. 9 p. m., urine contains traces of phthalein.

April 11. Abundant feces. *Phthalein 38-42 per cent.*

April 14. Dog appears quite well. *Phosphorous 5 mgs.* in oil given subcutaneously.

April 15. Dog appears well; weight 17 lbs. *Phthalein 0.1 gm.* intravenously. 4 p. m., urine contains trace of phthalein.

April 16. Feces fairly abundant. *Phthalein 33-35 per cent.* Urine is negative for phthalein.

April 20. Dog is in a good condition; weight 17½ lbs. *Phosphorous 7.5 mgs.* in oil given subcutaneously.

April 21. Broth culture (0.3 cc.) 24 hour growth of *B. coli* given intravenously.

April 22. Dog appears in a good condition and eats food; weight 16¼ lbs. Blood plasma appears normal, and clot is firm.

Plasma Lipase .10-.10 Initial alkalinity.
.60-.70 Butyric acidity.

.70-.80 Acid production.

12 m., *Phthalein 0.1 gm.* intravenously. 5 p. m., urine contains phthalein, ¾ per cent, and no bile.

April 23. Abundant feces. *Phthalein 25-32 per cent.*

April 24. *Phosphorous 7.5 mgs.* in oil given subcutaneously.

April 25. 11 a. m., *phthalein 0.1 gm.* intravenously; weight 15 lbs. 4 p. m., urine contains abundant phthalein 1 per cent, and bile pigments are present.

April 26. Abundant feces. *Phthalein 30 per cent.*

April 27. Dog found dead. Autopsy: Bilateral pleurisy with about 1 to 200 cc. of turbid exudate. The mediastinal tissues everywhere are œdematous. The lungs are clear. Other viscera are negative except the liver. The liver lobules are slightly conspicuous and the color suggests some fatty change. Microscopical section: Liver shows evidence of beginning cirrhosis. The connective tissue about the portal spaces is œdematous and contains numbers of wandering cells. There are clumps and strands of new formed fibroblasts, and in places indefinite buds suggesting the structures assumed to grow from the bile ducts or strands of liver cells. Phagocytes containing pigment debris are present in these areas. In some lobules polymorphonuclear leucocytes are quite numerous, suggesting a persistence of the infection produced by the injection of *B. coli*. The liver cell columns are somewhat distorted. The liver cells in general are pretty normal looking. Some of them contain small fat droplets, however, and the bile canaliculi as a rule are distended with yellow colloid material. There are no necroses. Wandering cells of the mononuclear type are pretty numerous everywhere. It is obvious that one is dealing here with a subacute infectious and toxic injury to the liver, bringing about progressive changes with proliferation of fixed tissue cells and definite suggestion of early cirrhosis. Death was due in great part to pleurisy and empyema with no evidence of grave liver insufficiency. It is probable that the colon bacillus, together with the phosphorous, were responsible.

TABLE VII. DOG 12-82.—HYDRAZINE AND PHOSPHOROUS POISONING.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 5	.100	22	+	Hydrazine.	17½	
8	.100	27	+	Do.	16½	
10	.100	42	1¼	....	16½	
14	....	..	..	Phosp. 5 mg.	...	
15	.200	35	Traces.	....	17	
20	....	..	..	Phosp. 7.5 mg.	...	
21	....	..	..	*B. coli.*	...	*B. coli* (0.3 cc. broth) intravenously.
22	.100	30	¾	....	16½	Lipase above normal.
24	....	..	..	Phosp. 7.5 mg.	...	
25	.100	30	1	....	15	Bile in urine.
27	Death.	..	..	....	...	Empyema - early cirrhosis.

The preceding experiment (Dog 12-82) shows the effect of hydrazine sulphate, which causes a drop in phthalein output, as in the case of other drugs which injure the liver. This drug causes a fatty change in the liver cell with little cell necrosis, and it is interesting that the lipase is normal at this time, although in fatal hydrazine poisoning the plasma lipase will be much increased. Phosphorous was then given, followed by a broth culture of *B. coli* to combine the action of the poison with an infection. Under certain conditions (Opie)[3] this will cause cirrhosis and such was found to be the case in this experiment, where autopsy showed connective tissue proliferation and some distortion of liver cell columns.

ECK FISTULA AND CHLOROFORM POISONING.

DOG 12-51.—Brindle bull, female; weight 27 lbs.

April 3. 12 m., *phthalein 0.2 gm.* intravenously. 5 p. m., urine contains traces of phthalein.

April 4. 11 a. m., feces abundant. *Phthalein 45 per cent.*

April 24. Dog in excellent condition; weight 26½ lbs. Operation as usual for *Eck fistula.*

April 25-27. Dog is well and eats.

April 28. Wound scratched open, but was closed again by deep sutures. This necessitated slow granulation.

May 9. Dog in excellent condition; weight 26¼ lbs. 11 a. m., *phthalein 0.2 gm.* intravenously. 4 p. m., urine contains phthalein 1 per cent. 6 p. m., urine contains no phthalein.

May 10. Feces are abundant. *Phthalein 45 per cent.*

May 17. Dog in a good condition; weight 29 lbs. 5 p. m., *phthalein 0.2 gm.* intravenously.

May 18. Urine of previous night contains 1 per cent phthalein. Abundant feces during forenoon. *Phthalein 43 per cent.*

June 2. 3 p. m., dog in excellent condition; weight 29 lbs. *Chloroform anæsthesia 2 hours.* The anæsthetic was poorly taken and a large amount of the drug given.

June 3. 12 m., dog is quiet, but does not look very sick. *Phthalein 0.2 gm.* intravenously. Blood plasma clots in normal time, but the clot is decidedly soft. Fibrinogen estimation 50 to 100 mgs.

Plasma Lipase .00- .10 Initial alkalinity.
1.30- .90 Butyric acidity.

1.30-1.00 Acid production.

5 p. m., urine by catheter contains bile and albumin. Phthalein 2½ per cent. Dog is bleeding from neck and the granulating abdominal wound, indicating a serious drop in fibrinogen content.

June 4. 10 a. m., dog found dead. Feces were abundant in the cage. *Phthalein 5 to 10 per cent* ± (color red and very difficult to read). Intestinal contents collected carefully. *Phthalein 6 per cent.* Gall bladder contains phthalein ½ per cent. Abdominal cavity contains about 200 cc. of bloody fluid, and there had been a good deal of bleeding externally as well as internally. Lungs showed numerous purple moist areas of bronchopneumonia. All the itssues showed icterus. *Liver* showed central necrosis. Microscopical section: The usual hyaline, central necrosis is present, involving about ¾ or more of every lobule. The liver cells about the portal spaces show fatty degeneration.

TABLE VIII. DOG 12-51—ECK FISTULA AND CHLOROFORM.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 3	.200	45	Traces.	....	27	Normal.
24	....	..	..	....	26½	Eck fistula produced.
May 9	.200	45	1	Eck Fistula.	26¾	
17	.200	43	1	do.	29	
June 2	....	..	..	Chlorof'm 2 hrs.	29	Anæsthetic poorly taken.
3	.200	7	2½	....	29	Bleeding; fibrinogen low.
4	Death.	6 (intestine).	..	....	...	Lipase 5 times normal.

ECK FISTULA.

DOG. 12-2.—Black and tan mongrel, female.

Feb. 1. Ether anæsthesia with operation as usual and production of the Eck fistula.

Feb. 2. Dog is recovering rapidly and drinks milk.

Feb. 3. Weight 13½ lbs.

April 1. 12 m., *phthalein 0.1 gm.* intravenously. Weight 15 lbs. 5 p. m., catheterised urine turns deep red on adding sodium hydroxide, showing considerable amount of phthalein.

April 2. 2 p. m., feces abundant. *Phthalein 17 per cent.*

April 8. 12 m., *phthalein 0.1 gm.* intravenously; weight 16 lbs. 4 p. m., urine contains phthalein, ¼ per cent.

April 9. Abundant feces. *Phthalein 20 per cent.*

April 15. *Phthalein 0.2 gm.* intravenously; weight 15½ lbs. 4 p. m., urine by catheter phthalein, 2 per cent.

April 16. 9 a. m., urine from cage contains phthalein in traces. 10 a. m., abundant fluid feces. *Phthalein 30 per cent.*

May 26. Dog in excellent condition and there has been steady improvement in general condition; weight 17 lbs. 11 a. m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains 0.1 per cent.

May 27. 2 p. m., abundant fluid feces. *Phthalein 36 per cent.*

May 30. 5 p. m., *phthalein 0.1 gm.* by *stomach tube.*

May 31. Night urine from cage contains no phthalein. Following the purge abundant feces were collected. Phthalein 45 per cent.

June 2. 11 a. m., *phthalein 0.1 gm.* by *stomach tube*, after a period of fasting for 24 hours, insuring practically an empty stomach.

June 3. 10 a. m., abundant fluid feces. Phthalein 58 per cent.

June 17. 10 a. m., *phthalein 0.1 gr.* intravenously; weight 17 lbs. 12 m., urine contains phthalein, 0.3 per cent. 4 p. m., urine contains phthalein, 0.1 per cent ±.

June 18. 10 a. m., abundant feces. Phthalein 40-41 per cent.

June 19. Abundant feces. Phthalein present only in faint traces.

June 21. Plasma Lipase .10-.10 Initial alkalinity.
.30-.30 Butyric acidity.

.40-.40 Acid production.

July 10. 10 a. m., dog appears well. 16¼ lbs. *Phthalein 0.1 gm.* intravenously. 2.30 p. m., urine, phthalein, 0.4 per cent. 3 p. m., urine by catheter. Phthalein 0.5 per cent. Purged.

July 11. Abundant feces. Phthalein 38-40 per cent.

TABLE IX. DOG 12-2.—ECK FISTULA.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Feb. 1	.....	.....	.....	.....	.....	Eck fistula produced.
Apr. 1	.100	17	++	Eck Fistula	15	
8	.100	20	¼	.....do	16	
15	.200	30	2	.....do	15½	
May 26	.100	36	0.1	.....do	17	
June 2	.100*	58	0	.....do	18	*Given phthalein by stomach.
17	.100	40	0.4	.....do	17	
July 10	.100	40	0.9	.....do	16¼	Dog well.

This experiment (Dog 12-2) shows how an Eck fistula dog may give a low phthalein output some weeks after the operation. These dogs often die during the first month with signs of obscure intoxication and perhaps suffer from hepatic insufficiency. This dog was in a rather poor condition for 2-3 weeks after the operation and then gradually and steadily improved in general health. It will be noted that the phthalein curve shows a steady rise in output. Phthalein is found in the urine in all Eck fistula dogs and we believe this indicates a certain type of liver injury; note also the increase in lipase. The preceding experiment (Dog 12-51) shows no difference in phthalein output before and after the Eck fistula, but it should be noted that the dog was in perfect condition and

gained weight after the operation. Fatal chloroform poisoning and Eck fistula showed the same reaction in every respect, as noted in preceding experiments (Chloroform).

ECK FISTULA OF ONE YEAR'S DURATION.

Dog 61.—Adult mongrel, female; weight 13 lbs.

May 25, 1912. Ether anæsthesia and an operation as usual. *Eck fistula* produced. Dog made a good recovery and has appeared normal during the past year.

April 5, 1913. Dog is normal and active; weight 13¼ lbs. 1 p. m., *phthalein 0.1 gm.* intravenously. 7 p. m., urine contains phthalein 0.3 per cent.

April 6. Abundant feces. *Phthalein ½ to ⅘ per cent.* Urine contains faint traces of phthalein.

June 18. Dog in excellent condition; weight 15 lbs. 12 m., *phthalein 0.1 gm.* intravenously. 3 p. m., urine by catheter contains 0.8 per cent.

June 19. Abundant feces. Phthalein 42 per cent.

June 20. Soft feces. Phthalein 6 per cent. *Phthalein total ⅘ per cent.*

Plasma Lipase .10-.10 Initial alkalinity.
.20-.15 Butyric acidity.

.30-.25 Acid production.

PASSIVE CONGESTION, ASCITES AND CHLOROFORM INJURY.

Dog 12-44.—Irish terrier, female; weight 13 lbs.

Jan. 21. Ether anæsthesia and operation (McClure). The vena cava between the diaphragm and heart was exposed and its lumen narrowed to an extreme degree by placing fine silk sutures going through and through the vein wall. The caliber of the vein at the end of the operation was estimated between 1 and 2 mm., the original diameter being about 8 mm.

Jan. 22-23. Dog recovering well from operation.

Jan. 27. Abdomen is somewhat swollen.

Jan. 31. There is well-marked œdema of the legs and abdominal wall; weight 19¾ lbs. Respiration is grunting and labored. 3 p. m., *phthalein 0.1 gm.* intravenously. 5 p. m., ascitic fluid 1000 cc. removed. Phthalein was absent.

Feb. 1. 3. p. m., 1500 cc. ascitic fluid removed. Phthalein not demonstrable in 500 cc.

Feb. 3. Subcutaneous œdema is diminishing; weight 18½ lbs. Collateral venous circulation is conspicuous over the abdomen.

Feb. 4. Weight 20 lbs., and dyspnœa is well marked, due to the pressure of the ascitic fluid. 1400 cc. pale, straw-colored fluid removed from abdomen.

Feb. 6. Weight 17 lbs. The subcutaneous œdema is clearing. The venous collaterals are very conspicuous. Ascitic fluid, 600 cc. removed.

Feb. 7. Weight 18 lbs. Ascitic fluid 1700 cc. removed.

Feb. 15. Weight 17 lbs. Dog has excellent appetite and appears in perfectly good health. Ascitic fluid 600 cc. removed.

Feb. 18. Abdomen is very tense; weight 19½ lbs. Ascitic fluid 1500 cc. removed.

Feb. 20. 1000 cc. ascitic fluid removed.

Feb. 28. Weight 18¼ lbs. Ascitic fluid 1800 cc. removed.

March 11. Ascitic fluid 900 cc. removed.

March 28. Ascitic fluid 1500 cc. removed. Weight after removal 14¾ lbs.

March 31. Weight 20¼ lbs. Ascitic fluid 2000 cc. removed. The œdema has reappeared over the abdomen, which is very tense.

April 1. 1 p. m., *phthalein 0.1 gm.* intravenously; weight 16½ lbs. 2.30 p. m., urine contains no phthalein.

April 2. Abundant fluid feces. *Phthalein 32 per cent.*

April 7. Ascitic fluid 2000 cc. removed.

April 12. Weight 21 lbs. Abdomen and hindlegs very œdematous. 12 m., *phthalein 0.1 gm.* intravenously. 3 p. m., urine contains no phthalein.

April 13. 12 m., feces abundant. *Phthalein 39-41 per cent.* Dog is quite dyspnœic, due to accumulation of fluid; weight 22½ lbs. Ascitic fluid 2000 cc. removed.

April 15. Ascitic fluid 2000 cc. removed.

April 22. Ascitic fluid 1000 cc. removed.

April 24. 600 cc. ascitic fluid removed. Spleen after removal of fluid is large, nodular and easily palpable. Liver can be palpated.

May 9. Weight 19½ lbs. Dog is in excellent condition; no subcutaneous œdema. Collaterals are very conspicuous over the abdomen. 11 a. m., *phthalein 0.1 gm.* intravenously.

May 10. Feces abundant. *Phthalein 53 per cent.*

May 13. Ascitic fluid 1000 cc. removed.

May 20. Abdomen is greatly swollen; weight 22¼ lbs. Ascitic fluid 3000 cc. removed. 3 p. m., *chloroform anæsthesia* for *two hours.*

May 21. 11 a. m., *phthalein 0.1 gm.* intravenously; weight 16 lbs. 12.30 p. m., urine contains phthalein, 0.1 per cent. 2.30 p. m., urine contains abundant phthalein, 2 per cent. 3 p. m., ascitic fluid 500 cc. removed. After centrifuging a clear amber fluid is obtained, which turns to a clear rose red on the addition of sodium hydroxide. It is obvious that the phthalein after chloroform poisoning has passed into the ascitic fluid, as well as into the urine. The ascitic fluid

Ascitic fluid Lipase .10-.10 Initial alkalinity.
.30-.30 Butyric acidity.

.40-.40 Acid production.

5 p. m., urine contains phthalein, 1 per cent. 9 p. m., urine contains phthalein, 1 per cent.

May 22. Abundant fluid feces. *Phthalein 19 per cent.*

May 23. 9 a. m., abundant feces. Phthalein 0. Urine contains phthalein ½ per cent and bile tests are positive. Dog is hungry and eats pretty well. 5 p. m., urine contains no phthalein, but plenty of bile pigments.

May 24. Urine contains traces of bile pigments. Dog appears quite normal. During this time there has been no accumulation of ascitic fluid.

May 26. Dog is active and hungry; weight 14¼ lbs. 11 a. m., *Phthalein 0.1 gm.* intravenously. 5 p. m., urine contains traces of phthalein.

May 27. Abundant feces. *Phthalein 30 per cent.*

May 28. Feces contain no phthalein.

June 9. Ascites beginning to reappear; weight 15¾ lbs. Collateral veins over abdomen are inconspicuous.

June 16. Weight 18 lbs. Dog is in a good condition.

June 17. 11 a. m., *phathaelin 0.1 gm.* intravenously; weight 18 lbs. 2.30 p. m., urine contains only the faintest trace of phthalein. 3.30 p. m., ascitic fluid 800 cc. removed. Centrifuging gives a clear amber colored fluid which undergoes no change on the addition of sodium hydroxide showing the absence of any phthalein.

Ascitic fluid Lipase .10-.05 Initial alkalinity.
.05-.10 Butyric acidity.

.15-.10 Acid production.

After removal of fluid, spleen is not as hard and easily palpable and liver cannot be felt.

June 18. No feces; and purgatives are vomited.

June 19. Abundant feces soiled with vomitus. *Phthalein 23 per cent.* It is possible that, due to the delay in purgation, there

had been some absorption of phthalein, but it is evident that the output is definitely diminished.

July 10. Dog well; good appetite. Ascitic fluid accumulates slowly and collaterals are conspicuous; weight 17½ lbs. 10 a. m., *Phthalein 0.1 gm.* intravenously. 2.30 p. m., urine contains mere trace of phthalein.

July 11. Abundant fluid feces. *Phthalein 43 per cent.*

July 21. Dog in good condition; 17 lbs. Sacrificed. Autopsy at once. The peritoneal cavity contains about 1700 cc. of ascitic fluid. The peritoneal cavity shows a little thickening of the serous coverings. The liver lobules are glued together by this same chronic inflammatory reaction undoubtedly associated with repeated tappings. Liver is enlarged, but has diminished in size considerably with the escape of blood. The gall bladder and bile ducts are quite normal. Bile escapes easily into the duodenum. On section the liver lobules have rather conspicuous centers, many of which contain blood. The spleen is but little increased in size. The other organs are normal. Kidneys show definite congestion of the cortex. The pleural cavity shows no adhesions except between the vena cava and the lung where the sutures are placed. The lumen of the vena cava measures 7-8 mm. in circumference when opened and is covered by a smooth, intimal lining. The sutures are embedded in the wall, causing little pouchlike bulgings just below the constriction. The vein below is slightly thickened.

Microscopical section: The liver is characteristic of passive congestion. There is central atrophy and fatty degeneration. Some of the lobules show almost complete disappearance of the parenchyma and here the walls of the sinusoids are thickened. There is a slight increase in connective tissue about many of the portal structures. The liver parenchyma as a rule about the margin of the lobule is quite normal.

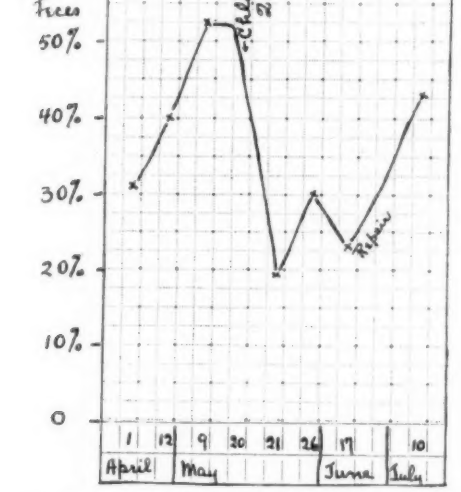

CHART III. DOG 12-44.—Passive Congestion and Chloroform Poisoning.

TABLE X. DOG 12-44.—PASSIVE CONGESTION AND CHLOROFORM.

Date.	Phthalein given.	Phthalein excreted.			Liver injury.	Weight.	Remarks.
		Feces.	Urine.	Ascites.			
		Per ct.	*Per ct.*	*Per ct.*		*Pounds.*	
Jan. 21	....	..	..	..	....	13	Vena cava partially occlud'd above diaphragm.
31	....	..	..	..	C. P. C.	19¾	Oedema of legs and abdomen.
31	.100	..	0	0	....	...	1000 cc. ascitic fluid removed.
Apr. 1	.100	32	0	..	C. P. C.	16½	Ascitic fluid removed freq'ntly.
12	.100	40	0	..	....	21	Oedema of legs.
May 9	.100	53	..	..	....	19½	
20	....	..	..	..	Chloroform 2 hrs.	22½	Ascitic fluid 3000 cc. removed.
21	.100	19	4½	+	....	16	Ascitic fluid 500 cc. Lipase increased in ascites.
26	.100	30	Trace.	..	....	14½	
Jun. 17	.100	23	Trace.	0	....	18	Ascitic fluid 800 cc.
Jul. 10	.100	43	Faint trace.	..	....	17½	

The preceding experiment is of considerable interest. Experimental passive congestion of the tissues and organs below the diaphragm can be easily produced by this method devised by R. D. McClure. It will be noted that the ascites did not appear at once and almost a week was required for advanced accumulation of ascitic fluid. The dog presented all the common signs of passive congestion of the abdominal viscera. In spite of this engorgement of the liver its functional capacity was but little impaired and the phthalein excretion rose as the collateral circulation became established and the liver adjusted itself to the changed conditions. This is in harmony with the observations of phthalein excretion in human cases.

Chloroform anæsthesia for two hours caused the usual sharp drop in phthalein output and, most interesting of all, the appearance of phthalein in the urine and ascitic fluid. Phthalein did not occur in the urine and ascites without acute liver injury. It is clear from this and other experiments that the presence of injured liver cells in the body modifies the phenoltetrachlorphthalein so that it can pass the kidney filter. It is obvious from this experiment that the phthalein is modified so that it can pass through the endothelial filter elsewhere than in the kidney and appears in the ascitic fluid. Lipase of the plasma is greatly increased during liver injury and the lipase of the ascitic fluid is about double normal during chloroform poisoning. The repair of the chloroform liver injury which advances so rapidly in normal dogs is found to be greatly delayed, probably due to the impaired circulation. Autopsy showed characteristic passive congestion of the liver with some central atrophy and connective tissue reaction.

HEMATOGENOUS ICTERUS AND ETHER ANÆSTHESIA.

Dog 12-1.—Fox terrier, female; weight 14½ lbs.

April 22. 12 m., dog is normal. *Phthalein 0.1 gm.* intravenously. 5 p. m., urine contals no phthalein.

April 23. Feces rather abundant. *Phthalein 38 per cent.*

April 25. Phthalein 0.1 gm. given by *stomach tube.*

April 26. Feces abundant. *Phthalein 60 per cent.*

June 3. 11 a. m., *ether anæsthesia* for 45 minutes. At the end of this time *phthalein 0.1 gm. given* intravenously. 5 p. m., catheterized urine contains no phthalein.

June 4. No feces; and dog vomits purgatives.

June 5. Feces are abundant. Phthalein 30 per cent. It is probable that this slight decrease below normal can be explained by the delay in purgation, which renders the amount extracted rather uncertain, because of possible absorption.

June 17. 12.30 p. m., dog is quite well; weight 15½ lbs. Intra-

venous injection of *hemoglobin solution* from normal dog. Normal washed red blood cells (85 cc.) were laked with 100 cc. distilled water and given intravenously. 2.30 p. m., dog vomits profusely and appears somewhat intoxicated. 3.30 p. m., second intravenous injection of *hemoglobin* solution exactly as at 12.30, the same amount being used. This was followed by an injection of *phthalein 0.1 gm.*, followed by 100 cc. of 0.9 per cent salt solution. Urine obtained at this time by catheter was a deep blood red color. Bile pigments positive (Huppert's test).

June 18. 10 a. m., urine was soiled with vomitus and feces, and showed traces only of phthalein. Soft feces and vomitus collected, *phthalein 30 per cent.* 11. a. m., urine (catheterized) contains hemoglobin and bile with considerable albumin. Phthalein negative.

June 19. Abundant feces Phthalein 10 per cent. Total phthalein 40 per cent.

July 26. Dog in good condition; weight 16 lbs. Sacrificed and autopsy at once. Thorax and heart are normal. Lungs show grayish nodules of organizing pneumonia (parasites). Liver appears normal in every way in gross. Bile can be easily squeezed through the bile papilla into the duodenum and the bile passages and gall bladder are quite normal. Microscopical section shows no abnormality. Other viscera normal.

The preceding history demonstrates two points: First, the administration of ether anæsthesia does not cause the appearance of phthalein in the urine as is the case with chloroform anæsthesia, where we know a definite liver necrosis is effected. This period of anæsthesia covers the time usually employed in the various operations upon the liver and gives control of this factor. Second, the intravenous injection of a solution of hemoglobin which leads to hematogenous icterus and heaping up of bile in the liver canaliculi, does not influence the output of phthalein. The liver is able to carry this extra load without giving any signs of impaired efficiency, as far as excretion of this drug is concerned.

Anemia and Hematogenous Icterus.

Dog 12-58.—Small fox terrier, female; weight 14¾ lbs.

April 18. 2 p. m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains no phthalein.

April 19. Feces are abundant. *Phthalein 35-40 per cent.*

May 12. Dog is normal; weight 16 lbs. Phenylhydrazine (1 per cent solution) 4 cc. given subcutaneously.

May 13. Phenylhydrazine 5 cc. subcutaneously. Hemoglobin (Sahli) 90-95 per cent.

May 14. Urine is high colored. Bile pigments negative. Phenylhydrazine 10 cc. subcutaneously.

May 15. Phenylhydrazine 10 cc. subcutaneously. Urine is high colored, but negative for bile pigments.

May 16. Phenylhydrazine 10 cc. subcutaneously. Urine contains traces of bile.

May 17. Urine contains large amounts of bile and is very dark. Dog appears rather sick; weight 14 lbs. 5 p. m., *phthalein 0.1 gm.* intravenously.

May 18. Dog shows slight icterus tint in skin. Urine of night contains very faint traces of phthalein. Hemoglobin 44 per cent. No feces.

May 19. 10 a. m., abundant feces but the extraction was very difficult and accurate reading was impossible. *Phthalein 30 per cent* ±.

May 20. Bile present in urine. Dog appears rather sick.

May 22. Dog is improving; hemoglobin 46 per cent; weight 13 lbs. 12 m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains traces of phthalein.

May 23. Abundant feces. *Phthalein 35 to 41 per cent.*

May 24. 10 a. m., more feces. Phthalein 2 per cent.

May 28. Dog appears normal. Hemoglobin 67 per cent. Phenylhydrazine (1 per cent solution) 10 cc. subcutaneously.

May 29 to June 4. Phenylhydrazine 10 cc. subcutaneously given each day.

June 5. Hemoglobin 27 per cent. Urine is free from bile. and very high colored; weight 14½ lbs. 12 m., *phathalein 0.1 gm.* intravenously. 3.30 p. m., urine contains no bile and a definite trace of phthalein. Phenylhydrazine given subcutaneously.

June 6. Abundant feces. *Phthalein 40 per cent.*

June 8. Phenylhydrazine 10 cc. given subcutaneously; weight 13¾ lbs.

June 9. Dog is active; weight 13½ lbs. Hemoglobin 25 per cent. 12.30 p. m., *phthalein 0.1 gm.* intravenously. 4 p. m., urine contains traces of phthalein and gives positive tests for bile pigments.

June 10. Abundant fluid feces. *Phthalein 55 per cent.* Urine contains bile pigments.

Table XI. Dog 12-58.—Anemia and Icterus.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Hemoglobin.	Weight.	Remarks.
		Feces.	Urine.				
		Per ct.	*Per ct.*		*Per ct.*	*Pounds.*	
Apr. 18	.100	40	0	....	..	14¾	Normal.
May 12	....	..	..	....	95	16	Phenylhydrazine daily May 12-16.
17	.100	..	Faint trace.	Icterus.	44	14	Bile in urine.
19	....	30±	..	do.	..	..	Delay in purgat'n.
22	.100	45	Trace.	....	46	13	Bile, traces in urine.
28	....	..	..	....	67	14	Phenylhydrazine daily May 28 to June 8.
Jun. 5	.100	40	..	Anemia.	27	14¼	Bile 0.
9	.100	55	Trace.	Icterus.	25	13½	Bile in urine.

Anemia and Hematogenous Icterus.

Dog 12-129.—Brindle bull dog, female; weight 28¼ lbs.

May 28. Hemoglobin (Sahli) 98 per cent. 5 p. m., *phthalein 0.2 gm.* intravenously.

May 29. Urine contains faint traces of phthalein. 3 p. m., abundant feces. *Phthalein 45 per cent.*

May 29-30. Phenylhydrazine (10 cc. of a 1 per cent solution) subcutaneously.

June 2. Phenylhydrazine 15 cc. subcutaneously.

June 3. Hemoglobin 60 per cent. Phenylhydrazine (15 cc.) subcutaneously.

June 4. Phenylhydrazine (15 cc.) subcutaneously; weight 24¼ lbs.

June 5. Hemoglobin 40 per cent. 15 cc. phenylhydrazine subcutaneously. 12 m., *phthalein 0.2 gm.* intravenously; weight 24 lbs.

June 6. Abundant feces. *Phthalein 41 per cent.*

June 8. Dog is rather weak and pale; weight 22 lbs. Phenylhydrazine 15 cc. subcutaneously.

June 9. Dog is very pale. *Hemaglobin 15 per cent.* Blood taken directly from vein and read in four volumes. 12 m., *phthalein 0.2 gm.* intravenously. 4 p. m., urine contains bile pigments. No albumin. *Phthalein 0.1 per cent* ±.

June 10. Night urine contains traces of phthalein and considerable bile pigments. Abundant fluid feces. *Phthalein 39 per cent.*

June 16. Dog has lost a great deal of weight and is not eating well. Hemoglobin 50 per cent; weight 17½ lbs.

June 17. 11 a. m., *phathalein 0.2 gm.* intravenously. 4 p. m., urine contains faint traces of phthalein.

June 18. Abundant feces. Phthalein 36-38 per cent.
June 19. More feces. Phthalein 10 per cent.
June 23. Dog is improving; weight 19½ lbs.

TABLE XII. DOG 12-129.—ANEMIA AND ICTERUS.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Hemoglobin.	Weight.	Remarks.
		Feces.	Urine.				
		Per ct.	*Per ct.*		*Per ct.*	*Pounds.*	
May 28	.200	45	Traces.	....	98	28¾	Normal.
Jun. 3	....	..	..	Anemia.	60	24¼	Phenylhydrazine daily May 29 to June 8.
5	.200	41	..	....	40	24	
9	.200	..	0.1±	Icterus.	15	..	Bile plus in urine.
10	....	39	+	do.	..	..	Dog pale and weak.
17	.200	..	+	....	50	17½	Emaciation.
18	....	37	..	....	..	..	Delay in excret'n.
19	....	10±	..	....	..	..	Dog improving.

The two preceding experiments (Dogs 12-58 and 12-129) were performed to throw some light on the question of hematogenous icterus and liver function. In these two experiments the blood was destroyed in vivo by means of phenylhydrazine given daily. The destruction must have been considerable, as the hemoglobin dropped from 98 per cent to 15 per cent and icterus was evident, with bile pigments abundantly present in the urine; yet the phthalein output in the feces remained at a normal level. A very slight fall in the second case could not have been recognized without an accurate normal base line, and we may assume that hematogenous icterus of a considerable degree does not impair liver function, as expressed by phthalein excretion.

The question of anæmia may be considered at this point, and it may be concluded that a secondary anæmia of moderate severity will not influence liver activity.

OBSTRUCTIVE ICTERUS.

DOG 12-138.—Young brindle bull, female; weight 24½ lbs.

June 9. 12 m., *phthalein 0.2 gm.* intravenously. 4.30 p. m., urine contains very faint traces of phthalein.

June 10. Abundant fluid feces. *Phthalein 4¼ per cent.*

June 16. Ether anæsthesia and operation with *ligation of common bile duct.*

June 17. Dog is in a good condition. Urine contains much bile pigment. 10 a. m., *phthalein 0.2 gm.* intravenously; weight 21¼ lbs. 12 m., urine by catheter contains bile pigments and phthalein 4½ per cent.

June 18. Night urine contains phthalein ½ per cent ±. Icterus is evident in the scleræ. The dog is lively and hungry.

June 19. Condition the same. Night urine contains phthalein 1 per cent ±. Bile is very abundant.

June 20. Soft clay feces. *Phthalein 0.* Urine contains much bile, no albumin and definite traces of phthalein. 12 m., urine by catheter contains no phthalein. Icterus seems to be less intense. *Phthalein 0.2 gm.* intravenously.

June 21. Night urine contains considerable phthalein. 9.30 a. m., fresh urine contains phthalein 0.8 per cent. 10 a. m., urine by catheter contains bile and phthalein 0.3 per cent. 11 a. m., soft pasty feces contain traces of phthalein and obviously the duct is cutting through and establishing the outflow from the liver.

June 22. Feces abundant. *Phthalein 12 per cent.* 12 m., urine by catheter contains only faint traces of bile and no phthalein. Icterus has disappeared.

June 23. Dog is hungry and appears normal; weight 22¼ lbs. Urine by catheter is free from bile and shows a faint cloud of albumin. Feces contain phthalein 2-5 per cent. 12.30 p. m., *phthalein 0.2 gm.* intravenously. 3 p. m., urine contains phthalein 0.4 per cent. 4 p. m., urine contains only traces of phthalein.

June 24. Abundant feces. *Phthalein 60-62 per cent.*

June 25. Urine and feces contain no phthalein.

July 22. 10 a. m., dog normal; weight 22½ lbs. *Phthalein 0.2 gm.* intravenously. 5.00 p. m., urine by catheter. Phthalein present in traces. Bile pigments absent.

July 23. Abundant fluid feces. *Phthalein 39 per cent.*

TABLE XIII. DOG 12-138.—OBSTRUCTIVE ICTERUS.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
June 9	.200	44	0	.....	24½	Normal.
16	.....	.....	.....	Icterus	.....	Bile duct ligated.
17	.200	.....	4½	.....do	21¼	Bile + + in urine.
18	.....	.....	¼	.....do	.....	Jaundice of sclerae.
19	.....	.....	1±	.....do	.....	
20	.200	0	0	.....do	.....	Jaundice less intense.
21	.....	Traces.	1	.....do	.....	Bile duct cut through.
22	.....	13	0	.....	22	
23	.200	4±	0.4	.....	22¼	Bile 0 in urine.
24	.....	60	0	.....	.....	
25	.....	0	0	.....	.....	Dog normal.
July 22	.200	39	Traces.	.....	22½	

The preceding experiment (Dog 12-138), as well as the following one (Dog 12-90), gives clear evidence that acute obstructive icterus caused by closing the common bile duct is associated with actual injury of the liver parenchyma. Other tests of liver function support this view. It will be seen that the phthalein escapes in the urine in obstructive icterus, but not in hematogenous icterus to any extent. This experiment shows very nicely how simple ligation of the common bile duct will cause obstruction for only about five days. The ligature cuts through and lies in the duct wall, but the ducts above may remain dilated and scar tissue formation may cause partial stricture (see below Dog 12-90). The abnormally high phthalein output following the last injection indicates a patent duct and probably a sweeping out of stagnant bile containing some phthalein of the previous injection.

OBSTRUCTIVE ICTERUS AND PHENYLHYDRAZINE POISONING.

DOG 12-90.—Small mongrel, male; weight 11 lbs.

April 6. Ether anæsthesia. Operation and *litigation of common bile duct.*

April 7-9. Bile is present in the urine.

April 10. Dog seems normal. There is no icterus and bile duct is probably cutting through. 3 p. m., *phthalein 0.1 gm.* intravenously; weight 11 lbs. 9 p. m., urine contains phthalein 0.3 per cent.

April 11. Urine contains faint traces of phthalein. Feces are abundant and fluid. *Phthalein 4.3 per cent.*

April 14. *Phenylhydrazine sulphate 0.1 gm.* given subcutaneously.

April 15. Dog appears rather sick; weight 10 lbs. 1 p. m., phthalein 0.1 gm. intravenously. *Phenylhydrazine 0.1 gm.* given subcutaneously. 5 p. m., urine soiled with a little vomitus. Phthalein 1½ per cent.

April 16. No feces. Urine plus vomitus phthalein, 4 per cent. 4 p. m., small amount of feces. Phthalein present in traces, 1 to 2 per cent ±.

April 17. Jaundice is evident in skin. Dog is quite dull. One soft stool. Phthalein present in mere traces.

April 18. Dog is somewhat better; weight 8½ lbs. Urine is rich in bile pigments and contains a trace of albumin. 2 p. m., *phthalein 0.1 gm.* intravenously. 4 p. m., urine contains traces of phthalein and much bile pigment.

April 19. Urine contains abundant phthalein, 2 per cent. One fluid stool. Phthalein 8 per cent.

April 22. Urine contains no bile and no albumin. Dog is much better; weight 9 lbs. 12 m., *Phthalein 0.1 gm.* intravenously. 5 p. m., urine contains phthalein, 2 per cent.

April 23. Abundant feces. *Phthalein 35 per cent.*

May 13. Dog is in good condition and weighs 11½ lbs. Sacrificed. Autopsy at once. Organs are normal except liver and bile passages. There are old adhesions around the bile duct between the duodenum and liver at the site of operation. Bile and hepatic ducts are dilated to about double normal size with definite stricture at the site of ligatures on the common duct. These ligatures have cut through and are situated in the wall of the duct, a part of the ligatures protruding into the lumen of the duct, which is narrowed at this place. The ligatures are crusted with salts and occlude the lumen in part, but a part of the obstruction is due to the narrowing effected by the new-formed scar tissue. The liver is practically normal in gross. Microscopical section shows practically normal parenchyma except for an occasional fat droplet in a liver cell. The portal tissues in places show a little increase in stroma and wandering cells. It is possible that with this narrowed duct the phenylhydrazine poisoning by the production of inspissated bile may have caused temporary complete obstruction, and that the great drop in output may have been due to this instead of profound liver injury.

TABLE XIV. DOG 12-90.—OBSTRUCTIVE ICTERUS AND PHENYLHYDRAZINE POISONING.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 5	.....	.....	.....	Icterus	11	Bile duct ligated.
7-9	.....	.....	.....	.....	.....	Bile + in urine.
10	.100	43	$\frac{3}{16}$	.....	11	Bile 0 in urine; duct cut through.
14	.....	.....	.....	Phenylhydrazine 0.1 gm.	.....	
15	.100	.....	1¼	.....do	10	Dog is sick.
16	.....	2	¼	.....	.....	Urine + vomitus.
17	.....	Traces.	.....	.....	.....	Bile ++ in urine.
18	.100	.....	Traces.	Icterus	8¼	
19	.....	8	2	.....do	.....	
22	.100	.....	2	.....	9	Dog is recovering. Bile 0 in urine.
23	.....	35	.....	.....	.....	
May 13	Sacrificed.	.....	.....	.....	11¼	Moderate stricture of bile duct; no icterus.

The autopsy findings in the preceding case (Dog 12-90) are of especial interest and suggest transient periods of complete obstruction associated with a narrowed common duct and inspissated bile due to liver injury (phenylhydrazine). The last injection of phthalein gave interesting information. The dog had no bile in the urine and seemed normal, yet the phthalein of the feces was below normal and the urine contained 2 per cent. This of itself spoke for liver injury and impairment which was shown by the autopsy.

ALEURONAT IN PORTAL VEIN.

Dog 12-77.—Fox terrier, male; weight 15 lbs.

April 18. 2. p. m., *phthalein 0.1 gm.* intravenously. 5 p. m., urine contains no phthalein.

April 19. Abundant feces. *Phthalein 39 per cent.*

May 21. Dog in perfect condition; weight 16¼ lbs. Ether anæsthesia and operation with injection into portal vein of a *1 per cent suspension of aleuronat*, made up with starch solution and sterilized. 5 cc. of this solution injected slowly in portal vein.

May 22. 12 m., *phthalein 0.1 gm.* intravenously. Dog looks quite ill. 6 p. m., *urine* contains *phthalein, ½ per cent* ±.

May 23. Feces are abundant. *Phthalein 41-45 per cent.* Urine contains no albumin, no phthalein, but small amounts of bile pigments.

May 24. 10 a. m., urine contains no albumin, and no bile pigments. Intravenous injection ½ cc. of 24-hour broth culture of *B. coli.*

May 28. Dog is active as usual; weight 16 lbs. 5 p. m., *phthalein 0.1 gm.* intravenously.

May 29. Urine contains no phthalein. Feces abundant. *Phthalein 45 per cent.*

June 4. Dog sacrificed in another experiment. Liver is practically normal in gross. Microscopical sections give no positive evidence of changes in the neighborhood of the portal structures, but it is very likely that repair following this relatively trivial injury would go on with such rapidity as to restore structures completely to normal within a period of two weeks.

LIVER INJURY BY CAUTERY.

Dog 12-60.—White bull-dog, female; weight 22 lbs.

April 1. 1 p. m., dog is quite normal. *Phthalein 0.2 gm.* intravenously. 4 p. m., urine is very abundant and contains faint traces of phthalein.

April 2. 2 p. m., abundant feces. *Phthalein 50-55 per cent.*

June 4. Dog is in excellent condition; weight 23¼ lbs. Ether anæsthesia with laparotomy and exposure of liver. Three lobes of the liver were cauterized deeply, using a large iron cautery. The necrosis was fairly extensive. After the operation, given ¼ grain of morphia.

June 5. 12 m., dog appears in good condition. *Phthalein 0.2 gm.* intravenously. 3 p. m., urine by catheter contains phthalein, 0.5 per cent. 5 p. m., urine contains phthalein, 0.1-0.2 per cent.

June 6. Abundant fluid feces. *Phthalein 33-35 per cent.*

June 7. 12 m., dog in good condition; weight 22¼ lbs. *Phthalein 0.2 gm.* intravenously. 2.30 p. m., urine contains much phthalein, 1-2 per cent. Bile pigments are present. 8.30 p. m., urine contains phthalein, 1-2 per cent ±.

June 8. 11 a. m., urine by catheter contains no phthalein, and small amounts of bile pigments. No feces obtained.

June 9. 10 a. m., abundant fluid feces. *Phthalein 40 per cent.* 3 p. m., more feces. Phthalein 0.

June 10. 4 p. m., dog appears normal; weight 22½ lbs. *Phthalein 0.2 gm.* intravenously.

June 11. No feces obtained.

June 12. Abundant fluid feces. *Phthalein 48-52 per cent.*

TABLE XV. DOG 12-60.—LIVER CAUTERY.

Date.	Phthalein given.	Phthalein excreted.		Liver injury.	Weight.	Remarks.
		Feces.	Urine.			
		Per cent.	*Per cent.*		*Pounds.*	
Apr. 1	.200	55	Faint trace.	.....	22	Normal.
June 4	.....	.....	.....	Cautery	.....	Deep cauterization of three lobes.
5	.200	35	0.6	.....	23¾	
7	.200	40	2+	.....	22¼	Bile in urine.
10	.200	52	.....	.....	22¾	Dog well.

LIVER INJURY BY CAUTERY.

Dog 12-137.—Small fox terrier, female; weight 13 lbs.

June 2, 12 m., ether anæsthesia and operation. Liver lobes burnt with actual cautery, three lobes being seared deeply. At the end of the operation *phthalein 0.15 gm.* intravenously. 5 p. m., urine contained traces of phthalein.

June 3. No feces.

June 4. Feces abundant. *Phthalein 15 per cent.* 12 m., *phthalein 0.1 gm.* intravenously; weight 11¾ lbs. 3 p. m., urine by catheter contained definite amounts of phthalein, giving a cherry-red solution on adding hydroxide. 5 p. m., urine by catheter contained phthalein.

June 5. Abundant feces. *Phthalein 48 per cent.*

June 6. Blood stained feces with some vomitus. *Phthalein 10 per cent.*

June 25. Dog sacrificed. Heart, lungs, spleen and kidney and intestinal tract normal. Dense adhesions about the liver, particularly over the areas which were cauterized. There is a definite cavity containing thick, shreddy, digested material, together with bits of necrotic liver parenchyma, whose wall is made up of dense granulation tissue measuring 3 or 4 mm. in thickness. This occupies a portion of one liver lobe. In places there has been marked constriction with distortion of the neighboring parenchyma. The liver lobules in general outside of the area of injury are normal, except for some central fatty degeneration. Microscopical section shows the wall of granulation tissue made out in gross. The liver lobules in association with this tissue are somewhat distorted, but the portal tissue is not markedly thickened. The lobules in general show a moderate grade of central fatty degeneration and leucocytes are fairly numerous in the capillaries. This change is more marked close to the liver scars. It is evident that a considerable amount of liver parenchyma had been destroyed by this procedure and replaced in part by inflammatory tissue, in which can be seen embedded the hyaline masses of necrotic parenchyma. A slight grade of bacterial infection is probably superposed, indicated by a considerable number of polymorphonuclear leucocytes. In places the dead liver tissue acts as a foreign body, causing the formation of syncytial masses of giant cells.

The three preceding experiments (Dogs 12-77, 60 and 137) illustrate a type of direct liver injury which is not due to any drug or poison capable of influencing the condition of other organs; for example, the kidneys. The injection of aleuronat into the portal vein will cause liver necrosis and we may imagine this process to have taken place in the first experiment. The phthalein output in the feces was not influenced by this minor liver injury, but phthalein appears in the urine, and it is impossible to attribute this to any kidney injury or to any process except actual liver injury.

Actual cauterization of the liver gives a massive destruction of liver parenchyma, such as might result in amebic abscess of the liver or extensive metastatic involvement in tumor growth. This procedure gives a definite fall in phthalein output in the feces and phthalein appears in the urine as a result of parenchyma injury.

PHTHALEIN IN URINE DEPENDENT UPON LIVER INJURY.

Dog 12-117.—Mongrel male, adult; weight 17¾ lbs.

May 16. 3 p. m., *chloroform anæsthesia 2 hours.*

May 17. 9 a. m., urine contains bile pigments. *Phthalein 0.2* gm. intravenously. 10 a. m., urine, 30 cc., contains faint traces of phthalein. 10.30 a. m., urine, 50 cc., contains phthalein 0.3 per cent. 11 a. m., urine, 10 cc., contains phthalein, 0.4 per cent. 11.30 a. m., *dog was transfused directly into Dog 12-118.* Then perfused with normal salt solution and these washings after removal of red blood cells and defibrination given also to Dog 12-118. These washings contain 2 mgs. of phthalein estimated from a fraction of the material.

Autopsy performed at once. All the tissue gave a bright red reaction with sodium hydroxide, indicating large amounts of phthalein. The contents of the intestine, washed out carefully, are rich in bile, but contain not a trace of phthalein, indicating a delay in excretion. Bile in gall bladder contained no phthalein. The mucosa of the gall bladder after washing gave a very deep pink color with sodium hydroxide, showing the presence of phthalein in the blood vessels of the mucosa. Liver shows little change in gross except conspicuous lobulations and increase in size. Under the microscope early liver necrosis is quite obvious, involving about three-fourths of every liver lobule. The liver was removed and ground up with sand with the formation of a uniform emulsion. To this was added a solution containing 200 mgs. of phthalein, and normal salt was added sufficient to make a fairly uniform paste. This mixture was allowed to stand in a water bath at 38° to 40° C. for three hours, used in Dog 12-119.

Dog 12-118.—Mongrel, male; weight 14 lbs. Ether anæsthesia and bleeding from femoral artery (225 cc.). Immediately after this bleeding direct transfusion from Dog 12-117 was done, using femoral artery to femoral vein. After the transfusion 300 cc. of the serum washings (Dog 12-117) were given intravenously by gravity. 12.25 p. m., urine by catheter. Phthalein 0. 4.20 p. m., urine by catheter. *Phthalein positive.* Definite rose red color on adding sodium hydroxide. 6 p. m., urine contains faint traces of phthalein.

May 18. Feces abundant. Phthalein 0. This dog received only a few milligrams of phthalein by means of this transfusion, as shown by the feces, which contain no demonstrable phthalein, yet the phthalein had been so changed as to escape through the kidney filter.

Dog 12-119.—Active fox terrier, female; weight 13¼ lbs. Intravenous injection of liver emulsion from Dog 12-117. The ground up liver mash plus 0.2 gm. phthalein incubated at 38-40° C. for three hours was made faintly alkaline with sodium hydroxide and squeezed through a cloth, centrifuged and filtered through cotton. 145 cc. of this thick, viscid filtrate given intravenously very slowly. The phthalein contained in this amount equaled .029 gm. 4 p. m., intravenous injection started. 5 p. m., injection completed. 4.45 p. m., urine by catheter contained hæmoglobin, which was removed by boiling and filtering. The clear amber filtrate on the addition of hydroxide showed a definite phthalein red color. Dog died during the night. Autopsy showed little of interest. The intestinal contents contained a few milligrams of phthalein, which could not be read accurately.

Dog 12-133.—Mongrel, female; weight 13½ lbs.

May 29. 3 p. m., *chloroform anæsthesia 2 hours.*

May 30. 10 a. m., *phthalein 0.2 gm.* intravenously. 11 a. m., Ether anæsthesia and bleeding from femoral. Blood defibrinated and clear amber serum obtained by centrifugalization. The dog was perfused with 1 litre of 0.9 salt. The washings collected and treated as the whole blood. First fraction yielded 200 cc. of clear amber serum, second fraction (washings) yielded 500 cc. pale serum. Both fractions given intravenously in Dog 12-134. Autopsy performed at once (Dog 12-133). Organs are normal except the liver, which shows an early necrosis involving about one-half of each liver lobule. Urine in bladder contains phthalein, 1 per cent.

Dog 12-134.—Fox terrier, female; weight 12 lbs.

May 30. 1 p. m., intravenous injection of blood serum obtained from Dog 12-133. This blood-phthalein mixture had circulated in contact with injured liver tissue for one hour. 2.15 p. m., urine in a clean cage contained traces of albumin. 2.45 p. m., urine by catheter 30 cc. Phthalein definite rose red with sodium hydroxide.

May 31. Feces collected. Phthalein 3 per cent indicating that the amount of phthalein introduced intravenously in this dog did not exceed 20 milligrams and probably not much over 10 milligrams.

The preceding group of experiments (Dogs 12-117 and 12-133) give the final proof that injured liver cells modify the phthalein in the blood stream so that the drug appears in the urine in appreciable amounts, which does not occur with a normal liver. Circulation of phthalein for one hour in contact with an injured liver (chloroform) is followed by the appearance of phthalein in this dog's urine, but most important of all this blood phthalein mixture when given to a normal dog intravenously is followed by the appearance of phthalein in the normal dog's urine. This rules out any secondary effect of the liver poison upon the kidney.

Discussion.

The normal output of phenoltetrachlorphthalein in the feces of dogs is remarkably constant. From a study of the tables above it will be found that the normal feces output rarely falls below 40 per cent and rarely exceeds 50 per cent—the usual normal being about 45 per cent. It will be seen that the individual normal variation is even less, and by careful work it is easy to fix the normal base line of any given dog with considerable accuracy. This enables one to make careful study of small fluctuations in phthalein output and this will be of interest in many forms of experimental work with hepatic function.

The drop in phthalein output appears to run parallel to the amount of parenchyma injury. Following a short chloroform anæsthesia or the injection of small doses of hydrazine or phosphorous there is a small drop in phthalein output, while after a grave injury or one sufficient to cause death the phthalein curve falls to zero. The mechanism of liver repair is familiar (Whipple and Sperry)[4] and with this repair the phthalein output returns to normal. In some instances following liver repair the output may rise above normal (Dog 12-53), as has been reported in earlier work with blood fibrinogen (Whipple and Hurwitz).[5] This may speak for a period of hyperactivity of these newly formed liver cells. After a trifling injury to the liver (*e. g.*, a small dose of phosphorous) there may be a period of *hypersecretion* of phthalein, and we may imagine the drug acting as an irritant to the secreting parenchyma—a stage perhaps preceding that of actual cell injury or necrosis.

Passive congestion, when of moderate degree, will cause little impairment of function and show a normal output, but during periods of marked circulatory embarrassment the liver will not be able to function properly and the output drops. The Eck fistula liver may present a normal output or show considerable impairment and this variation will parallel the general condition of the animal. The explanation of this variation in dogs with Eck fistulæ is not clear, in that we do not understand the causation of the liver degeneration which is usually present in such animals dying with symptoms of intoxication. It is well to bear in mind how much change in the hepatic circulation can be effected without impairing the dog's health or influencing the phthalein curve.

Destruction of the liver parenchyma by the actual cautery will cause a prompt drop in phthalein output, and it is probable that the presence of this injured tissue and the inflammatory reaction more than the actual loss of parenchyma is responsible for the drop in the curve. This experiment simulates in a way the human cases of amebic abscess or even metastatic tumor growth in the liver, which are associated with considerable parenchyma destruction.

Delay in phthalein excretion can be demonstrated following acute liver injury, but can be of little value in work with the feces. In experiments where the dogs are sacrificed a few hours after chloroform injury and phthalein injection, it will be found that the bile is free from phthalein or contains but traces, whereas it is known that normally the drug appears in the bile within fifteen to thirty minutes.

The urine contains no phthalein or at most a trace (faint rose red with alkali) following injection of the amounts of phthalein employed in normal dogs. After acute liver injury of any type (chloroform, phosphorous, aleuronat, cautery) the urine collected in the first six hours will contain 0.2 to 8.0 per cent. It seems that the injured liver cells modify the phthalein so that it can pass the kidney filter; moreover, so that it can escape into the ascitic fluid of a dog with passive congestion. This is not due to any kidney injury, as would be indicated by the liver cautery experiments, but is proved conclusively by the transfusion of blood containing phthalein from a dog with liver injury into a normal animal. In spite of the minimal amount of phthalein received by the normal dog, the urine shows its escape through the kidney.

Obstructive icterus of any considerable degree makes this test of little value. It will give some evidence of the amount of acute liver injury which may be present, depending upon the amount of the drug escaping in the urine. Some of the drug will be stored in the bile, but the greater part will be changed to some non recognizable form which cannot be recovered from the body. Hematogenous icterus and anemia of considerable severity do not modify phthalein excretion by the liver.

Chronic liver injury will offer more difficulties of interpretation, for we cannot expect to diagnose liver scars by means of any test unless the scars are of sufficient size and number to impair its functional activity. A cirrhotic liver like a diseased heart may have periods of normal activity, when it is quite capable of carrying on its normal work. The reserve force of an organ is always considerable and after an injury has healed, even perhaps leaving considerable scar tissue, the parenchyma may be able to function properly and sufficiently

for the bodily needs. Under such circumstances one cannot demand too much from any functional test, as the drug excretion may be normal, even though the liver be working close to its limit capacity and be liable to a period of broken compensation at any moment. There is a great need of accumulated data bearing on these points, careful clinical and anatomical study of cases with various types of liver lesion. Attention should not be limited to one test, but all forms of liver tests should be employed and compared with the clinical and anatomical findings.

This test promises much in the study of derangements of the hepatic parenchyma, physiological and anatomical. Its great value is that it may give some *quantitative values* concerning liver injury, and for this reason, with accumulated experience, may be of value to the clinician. It surely will be of considerable value in various experimental studies concerned with liver function and impairment. We hope to report further work along these lines in the near future.

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A TEST FOR HEPATIC INJURY: BLOOD LIPASE.

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Work with this ferment lipase was first started in this laboratory in connection with a study of acute hemorrhagic pancreatitis. It was hoped that the ferment might appear in the urine after experimental pancreatitis and be of diagnostic value. Hewlett [1] had noted the appearance of this ferment in the urine of animals with acute pancreatitis, and Opie [2] had recorded its presence in a human case. It was thought that the fat necroses which are so constant in this disease were produced by this ferment and that it might escape in the urine under these conditions.

A routine study of dogs' urine convinced us that small amounts of lipase (.05 to .20 cc.) were present at times normally. It was also found that after acute pancreatitis had been produced by the injection of bile into the duct or otherwise, lipase did appear in the urine promptly. The lipase content sometimes rose as high as 0.50-.60 cc. in the first hour after the operation, with a rapid fall to .10 cc. or even zero in six hours. Small amounts of lipase sometimes persisted in the urine for a day or so after the operation, but this seemed to bear no relation to the extent of pancreatic injury.

It was soon found that lipase appeared in the urine whether the pancreas was injured or not, and it became apparent that the anæsthesia was the sole cause for the sudden appearance of this ferment in the urine. Ether or chloroform will cause its appearance in the urine, but it is to be kept in mind that ether causes no rise in blood lipase, as it causes no liver necrosis. Chloroform, as is noted below, causes a marked and prompt rise in blood lipase associated with liver injury. The blood and urine lipase are in no way parallel, as lipase appears in the urine, especially during the first six hours after anæsthesia, while the plasma lipase reaches its maximum in 6-12 hours and remains high for two days or longer. We are familiar with the reports of other workers who have studied the lipase in the blood, but they have come to no very definite conclusions. Some workers claim that lipase is absent from blood serum, but they are in the minority.* Bauer [3] describes small fluctuations in serum lipase in cases of tuberculosis, cancer and syphilis, but gives no careful anatomical study. Von Hess [4] finds the serum lipase to be uninfluenced by pancreatic extirpation, peritonitis, hyperthyroidism, thyroidectomy and ether anæsthesia. Serum lipase is not activated by bile salts.

In the experiments tabulated below very little detail is noted, but the lipase content is recorded in some of the experiments reported in full in a preceding communication (Whipple, Peightal and Clark).[5]

METHOD.

The method (Loevenhart) for determining lipase in any of the body fluids may be outlined as follows: Four tubes are prepared, each containing 1 cc. plasma, serum, etc., diluted with 4 cc. distilled water and 0.3 cc. toluol added. To two of the tubes is added .26 cc. ethyl butyrate, the other two serving as controls. The tubes are all corked, shaken and incubated at 38° C. for 18 to 24 hours. They are cooled in ice water, three drops of azolitmin added as an indicator and titrated in pairs to a neutral reaction, using 1-10 normal acid and alkali. The two control tubes give the serum alkalinity to this indicator as about 0.1 cc. 1-10 normal acid. The butyrate tubes give the amount of acid production above the neutral point, and the sum represents the total acid production or lipolytic activity. This represents the amount of butyric acid which has been set free by the ferment, and it is estimated always in term of 1-10 normal acid. This may be spoken of as the blood lipase, serum or plasma, as the case may be.

It will be found that there is little difference between the plasma and serum lipase, the latter being a little higher because of the dilution of the plasma by the oxalate. The blood obtained from the dogs was almost always removed from a vein during life. Many of the specimens of human blood were obtained during life

* A general presentation of the work done upon the ferment lipase will be found in the Handbuch der Biochemie, Oppenheimer, 1909.

and often shortly after death before the blood had clotted. Blood from the autopsy cases was usually drawn directly into oxalate by a trocar in the heart.

TABLE I.—LIVER NECROSIS IN DOGS—CHLOROFORM ANÆSTHESIA.

No.	Acid production.	Alkalinity.	Butyric acidity.	Period following liver injury.	Remarks.
Co–9	.35– .35	.10–.10	.25– .25	.....	Normal.
Co–9	.45– .50	.10–.10	.35– .40	30 minutes...	Chloroform 2 hrs.
Co–9	1.70–1.50	.0–.0	1.70–1.50	14 hrs.....	Do.
89	.30– .35	.10–.15	.20– .20	.....	Pregnant.
89	.35– .40	.10–.10	.25– .30	End of anaesthesia.....	Chloroform 2 hrs.
98	.30– .25	.10–.10	.20– .15	.....	Normal.
98	.35– .35	.10–.10	.25– .25	End of anaesthesia.....	Chloroform 2 hrs.
98	.80– .85	.10–.10	.70– .75	3 hrs.....	Do.
98	.95–1.00	.10–.10	.85– .90	5 hrs.....	Do.
C–19	.35– .40	.05–.10	.30– .30	.....	Normal.
C–19	.42– .40	.10–.10	.32– .30	End of anaesthesia.....	Chloroform 2½ hrs.
C–19	1.60–1.40	.10–.10	1.50–1.30	5½ hrs.....	Do.
C–19	1.50–1.55	.10–.10	1.40–1.45	24 hrs.....	Do.
C–19	1.45–1.45	.10–.10	1.35–1.35	2 days.....	Do.
C–19	1.05–1.05	.15–.10	.90– .95	4 days.....	Do.
C–9	.30	.05	.25	.....	Normal.
C–9	.60– .65	.10–.10	.50– .55	2 hrs.	Chloroform 2 hrs.
C–9	1.65–1.80	.15–.10	1.50–1.70	2 days.....	Do.
B–13	1.50–1.80	.10–.10	1.40–1.70	2 days.....	Do.
B–13	.35– .40	.10–.15	.25– .25	8 days.....	Chloroform 2 hrs. Complete rep'r.
12–48	1.10–1.40	.10–.10	1.00–1.30	2 days.....	Chloroform 1¾ hrs.
12–48	.70– .60	.10–.10	.60– .50	24 hrs.....	Chloroform 1 hr.
12–78	1.10–1.45	.10–.10	1.00–1.35	24 hrs.....	Chloroform 2 hrs.
12–141	.55– .80	.15–.15	.40– .65	4 days.....	Do.
12–141	.40– .50	.10–.10	.30– .40	5 days.....	Bit of liver removed on 2d day. ⅜ lobule shows hyaline necrosis.
11–141	.30– .30	.10–.10	.20– .20	6 days*....	

*Repair complete.

Table I gives a sufficient number of observations to show the plasma lipase reaction following a chloroform anæsthesia, which is known to produce a uniform central hyaline liver necrosis involving from two-fifths to three-fifths of each liver lobule. The lipase content rises rapidly after the end of anæsthesia, reaching a maximum in six to twelve hours and remaining pretty constant and uniformly elevated until the end of forty-eight hours, when a fall begins and returns to normal on the sixth day or a little later. This parallels the process of repair which goes on so rapidly in the liver during the week following chloroform injury. It will be seen that a shorter narcosis will cause a smaller rise in lipase (see Dog 12-48) when the experiments are done upon the same animal to exclude any individual variations. It is of importance that the individual variations are but slight. We may conclude that in general a high lipase indicates considerable liver necrosis and lesser amounts of necrosis will be associated with minor rises in plasma lipase. It is probable that some other factors may influence this reaction, and these will be discussed later.

Chart I shows in a diagrammatic way the combined observations of Table I. When the chloroform poisoning is fatal, leading to death on the third or sixth day, the lipase curve usually remains high as on the second day, but it may fall somewhat as in phosphorous poisoning. (See Chart III.)

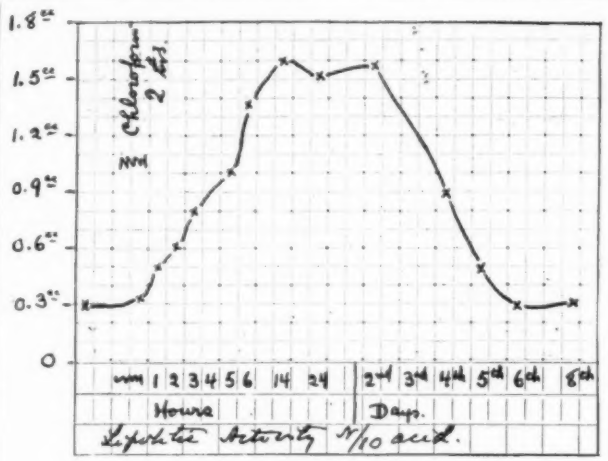

CHART I.—PLASMA LIPASE CURVE, LIVER INJURY, CHLOROFORM.

TABLE II.—PHOSPHOROUS AND HYDRAZINE POISONING IN DOGS.

No.	Acid production.	Alkalinity.	Butyric acidity.	Period following liver injury.	Remarks.
12–88	1.55–1.20	.10–.10	1.45–1.10	24 hrs	Phosphorous subcutaneously.
12–88	.70– .60	.10–.10	.60– .50	3 days.....	Phosphorous subcutaneously.
12–88	.75– .70	.10–.10	.65– .60	6 days, death.	Phosphorous liver necrosis and fat.
12–82	.70– .80	.10–.10	.60– .70	2 days.....	Phosphorous; death 7th day.
12–80	.85– .75	.10–.10	.75– .65	6 days, death.	Phosphorous liver.
12–82	.20– .20	.10–.10	.10– .10	2–3 days.....	Hydrazine sulphate, 2 small doses.
12–87	.65– .65	.10–.10	.55– .55	2 days, death.	Hydrazine sulphate, lethal dose. Peripheral liver necrosis and fat.

TABLE III.—ECK FISTULA IN DOGS.

No.	Acid production.	Alkalinity.	Butyric acidity.	Period following liver injury.	Remarks.
12– 2	.40– .40	.10–.10	.30– .30	5 months....	Eck fistula.
61	.30– .25	.10–.10	.20– .15	13 months...	Do.
12–51	1.40– .90	.10–.10	1.30– .80	24 hrs.....	Eck fistula plus chloroform 2 hrs. ¾ liver lobule hyaline necrosis.
C–89	.40– .35	.10–.10	.30– .25	.....	Slight icterus.
C–89	1.70–1.60	.05–.10	1.65–1.50	6½ hrs.,death.	Eck fistula plus hepatic artery ligation.
C–95	.20– .25	.10–.10	.10– .15	.....	Normal.
C–95	1.60–1.65	.10–.10	1.50–1.55	5½ hrs.,death.	Eck fistula plus hepatic artery ligation.

Table II shows the lipase reaction following phosphorous and hydrazine poisoning. After phosphorous has been given in oil subcutaneously the maximum rise of the lipase curve is found after twenty-four hours, when it closely parallels the chloroform curve. On the following days, even in fatal cases,

the lipase falls to about one-half its highest level, but still remains well above normal until death. It is well known that in chloroform poisoning the amount of cell necrosis is very much greater than in phosphorous poisoning. The former poison attacks the nuclei of the liver cells, causing hyaline necrosis, and the latter injures the protoplasm, causing much fatty degeneration, but scattered liver cells show hyaline necrosis. This difference in amount of actual cell necrosis may explain some of the points of difference in the lipase curves.

Table III shows the plasma lipase of dogs with Eck fistulæ of various duration. Shortly after operation an Eck fistula dog may show a very high plasma lipase and such dogs may show advanced liver degeneration, but rarely actual necrosis. When the fistula has been present for many months the lipase tends to return to normal, but may still show periods of elevation. The reaction to chloroform poisoning is identical with that of a normal dog. When an Eck fistula has been performed and the hepatic artery, with all its branches, ligated the dog will always die in 5-7 hours with characteristic symptoms. Under such conditions a very little blood can trickle through the liver by means of collaterals, but not sufficient to keep the cells alive, and they show evidences of autolysis at the end of the experiment. This small stream of blood which bathes these dying liver cells returns to the general circulation and is responsible for the great rise in plasma lipase found at the end of such experiments. Here, as elsewhere, the rise of blood lipase is associated with dead or injured liver cells in contact with the blood stream. In this instance the liver cells have been killed not by any poison, but by lack of blood.

TABLE IV.—OBSTRUCTIVE ICTERUS AND ACUTE HEMORRHAGIC PANCREATITIS IN DOGS.

No.	Acid production.	Alkalinity.	Butyric acidity.	Period following liver injury.	Remarks.
C-3	.45-.55	.10-.10	.35-.45	7 days.....	Bile duct ligated.
86	.30-.35	. 0-.05	.30-.30	5 days.....	Bile duct ligation; rupture & peritonitis.
B-42	.40-.35	.05-.05	.35-.30	14 days.....	Do.
B-3	1.20-.95	.10-.10	1.10-.85	8 days.....	Bile duct ligated; cholangitis; hyaline liver necroses.
S-4	.30-.35	.10-.10	.20-.25	24 hrs.....	Closed duodenal loop.
S-12	.25-.20	.10-.10	.15-.10	48 hrs.....	Closed duodenal loop; intoxication; corresponds to acute intestinal obstruction.
B-1	.20-.20	.10-.10	.10-.10	2 days.....	Acute hemorrhagic pancreatitis.
B-5	.30-.30	.10-.10	.20-.20	2 days.....	Acute hemorrhagic pancreatitis.

Table IV indicates the small rise in lipase which is associated with acute obstructive jaundice. Even when the bile duct ruptures with escape of bile into the peritoneal cavity with developing secondary peritonitis, the lipase shows but little rise above normal. However, if there is a *cholangitis*, with hyaline necroses and liver injury, the lipase shows a great rise above normal, very much as in chloroform liver necrosis.

Acute hemorrhagic pancreatitis with profound injury and necrosis of the parenchyma shows a normal plasma lipase. As reported in a recent article on Hemorrhagic Pancreatitis (Whipple and Goodpasture)[7] the peritoneal exudate contains the same amount of lipase as does the plasma, except in the last stages of a fatal case, where it may be assumed that the processes of exudation and neutralization are at a standstill.

Closed duodenal loops in dogs cause acute intoxication and death, which is identical with or very similar to the intoxication and death of acute intestinal obstruction. Under such circumstances the lipase of the blood is quite normal.

TABLE V.—ECLAMPSIA AND TOXÆMIAS OF PREGNANCY.

No.	Acid production.	Alkalinity.	Butyric acidity.	Autopsy liver.	Clinical diagnosis.	Remarks.
3672....	1.85-1.60	.10-.10	1.75-1.50	Massive portal necroses.	Eclampsia	Serum
3672....	1.80-1.70	.10-.10	1.70-1.60	.... do.....	.... do.....	Plasma.
3883....	.85-1.15	.10-.15	.75-1.00	.....	.... do.....	March 3, convulsions.
3883....	1.10-1.40	.10-.10	1.00-1.30	Many portal necroses.	.... do.....	March 4, death.
3748....	.45-.50	.05-.05	.40-.45	Portal necroses.	.... do.....	Death 8 hrs. after first convulsion.
3963....	.25-.25	.10-.10	.15-.15	.....	.... do.....	July 11, convulsions.
3963....	.20-.15	.10-.05	.10-.10	Very few portal necroses.	.... do.....	July 13, death.
5442....	1.30-1.25	.20-.20	1.10-1.05	.....	.... do.....	Antepartum. Recovery 16th day.
5465....	.60-.50	.10-.10	.50-.40	.....	.... do.....	Antepartum. Recovery 21st day.
5477....	.20-.20	.07-.05	.43-.15	.....	.... do.....	Intrapartum. Recovery 2d day.
5224....	.50-.55	.10-.15	.40-.40	.....	.... do.....	Postpartum.
5358....	.20-.20	.10-.10	.10-.10	.....	.... do.....	Antepartum. Chronic nephritis.
5312....	.30-.30	.10-.10	.20-.20	.....	Preeclamptic toxaemia.	No convulsions. Recovery 2 weeks.
5805....	.30-.30	.10-.10	.20-.20	.....	Eclampsia (?).	Convulsions, epilepsy.
5867....	.35-.35	.15-.25	.20-.20	.....	Eclampsia (?).	Chronic nephritis, final diagnosis.
5981....	.30-.30	.10-.15	.20-.15	.....	Eclampsia (?).	Chronic nephritis, final diagnosis.
3677....	.25-.30	.10-.10	.15-.20	Fatty degeneration.	Toxaemia.	
3667....	.30-.30	.10-.10	.20-.20	Marked fatty degeneration.	Vomiting of pregnancy.	

Table V is of particular interest, as it deals with eclampsia and various toxæmias of pregnancy. It may be admitted that at present there is a certain degree of confusion in this group of interesting diseases. Eclampsia is variously defined, but we wish to limit the term to that particular intoxication usually associated with convulsions, which presents characteristic hemorrhagic portal liver necroses. If this point is insisted upon, the diagnosis can never be absolutely certain unless death supervenes and a complete autopsy reveals the characteristic lesion.

The first four cases in the table were clinically typical eclampsias and the first two showed a very high blood lipase associated with a marked grade of portal hyaline necrosis. The third case shows a definite rise in lipase above normal, and liver necrosis was less marked. The fourth case shows a normal lipase and autopsy showed only a very few small portal

necroses. These were found only after very careful search in gross and by making a large number of microscopical sections. The areas involved were all situated at the hilum of the liver close to the large vessels and would have been missed completely by the usual routine examination and sections.

The next five cases of clinical eclampsia are of interest. Three show lipase much above normal, especially the cases with grave intoxication and slow recovery, and we may safely assume that liver necrosis was present in all. One case of intrapartum eclampsia showed a normal lipase and made a complete recovery by the second day. The last case was complicated with chronic nephritis and there may be some doubt about the diagnosis, as the lipase was normal.

Several cases of suspected eclampsia showed normal lipase and turned out to be chronic nephritis and even epilepsy in one instance.

The toxæmias and vomiting of pregnancy show normal lipase and the liver changes are limited to central fatty degeneration without liver necrosis.

From this analysis it is clear that the toxæmias of pregnancy, which have very high lipase, always present liver necrosis, and if peripheral the diagnosis of eclampsia is inevitable. Certain cases of eclampsia which are associated with very small and widely scattered portal necroses may show a normal lipase. The test is of value in differentiating various types of intoxication in pregnancy from true eclampsia.

TABLE VI.—LIVER NECROSIS IN HUMAN CASES.

No.	Acid production.	Alkalinity.	Butyric acidity.	Autopsy liver.	Clinical diagnosis.	Remarks.
3993....	.30–.30	.10–.10	.20–.20	.....	Cancer. Liver metastases.	April 4.
3993....	.60–.60	.10–.10	.50–.50	Cancer; central necrosis and fat.	.....	April 9, death.
3939....	.80–.80	.10–.10	.70–.70	Focal necroses.	Septicaemia. Nephritis.	
3726....	1.00–1.20	.10–.10	.90–1.10	.....	Chloroform poisoning.	Chloroform 1½ hrs. 4th day, previously.
3726....	1.00–1.15	.05–.05	.95–1.10	Central necrosis.	.... do.....	6th day, death.
3320....	.45–.40	.15–.15	.30–.25	Few central necroses.	Leukaemia.	
3680....	.80–.75	.10–.15	.70–.60	½ central necrosis.	.... do..... ...	Very acute.
3702....	.50–.55	.10–.10	.40–.45	Focal necroses.	Lobar pneumonia.	
3724....	.85–.75	.05–.05	.80–.70	⅓ central necrosis.	Aplastic anemia.	Necrosis missed in routine study.
3688....	.55–.45	.10–.10	.45–.35	Few hyal. liver cells.	Acromegaly. Infection.	Liver very fatty.

Table VI presents a group of cases in which liver necrosis was found at autopsy or in the routine histological study of the liver. It will be seen that the blood lipase is always above normal and often three to five times normal. The highest reading is in a case of late chloroform poisoning with death on the sixth day, in which case the liver necrosis involved a great part of every lobule. The readings in this case are exactly like those in the chloroform poisoning in dogs (Table I).

Table VII shows that chronic liver disease or icterus may be associated with a normal or even subnormal lipase. When

TABLE VII.—LIVER CIRRHOSIS AND ICTERUS IN HUMAN CASES.

No.	Acid production.	Alkalinity.	Butyric acidity.	Autopsy liver.	Clinical diagnosis.	Remarks.
3616....	.15–.20	.05–.05	.10–.15	Hemochromatosis.	.....	Cirrhosis and hemorrhage.
3485....	.40–.45	.10–.10	.30–.35	Many focal necroses.	Cirrhosis.	Icterus.
3634....	.10–.12	.05–.05	.05–.07	Focal necroses.	.... do.....	
Neizer..	.20–.25	.10–.10	.10–.15	.....	.... do.....	No clinical symptoms.
B. V....	.15–.10	.10–.05	.05–.05	.....	.... do.....	Marked toxaemia and icterus.
3665....	.20–.20	.10–.10	.10–.10	Slight cirrhosis.	Chronic nehpritis.	Passive congestion.
Jones ..	.20–.20	.10–.10	.10–.10	.....	Icterus 18 mos.	Tumor head of pancreas. Operation.
White .	.20–.20	.10–.10	.10–.10	.....	Icterus 4 mos.	Cancer head of pancreas. Operation.
3754....	.25–.20	.05–.05	.20–.15	Cancer: liver necroses.	Cancer icterus	Liver necrosis. No cellular reaction.
Woods .	.15–.15	.10–.10	.05–.05	.....	Icterus 3 mos.	Gall stones.

liver necrosis is combined with either of the above we may expect to see a definite rise in lipase, but this is not invariable. It is probable that chronic disease of the liver affects in some way this reaction against necrosis and injury which in the normal liver so constantly brings about the over-production or escape of lipase. One case (3754) with long-standing icterus, presents rather extensive hyaline central necrosis and normal lipase. It is of interest that although the necrosis obviously had been present over twenty-four hours, there had not been the slightest cellular reaction nor cell digestion which in most cases proceeds with great speed.

TABLE VIII.—NEPHRITIS. BLOOD DISEASES AND DIABETES.

No.	Acid production.	Alkalinity.	Butyric acidity.	Autopsy liver.	Clinical diagnosis.	Remarks.
3700....	.15–.20	.0–.0	.15–.20	Slight fatty degeneration.	Acute nephritis.	
3907....	.10–.10	.10–.10	.0–.0	Slight fatty degeneration.	Nephritis.	Subacute endocarditis and nephritis.
3665....	.20–.20	.10–.10	.10–.10	Slight cirrhosis.	Chronic nephritis.	Passive congestion.
Schwab .	.25–.25	.15–.15	.10–.10	.....	.....	Hemorrhagic disease.
Shema .	.20–.25	.05–.10	.15–.15	. ...	Chronic leukaemia.	Hemorrhagic disease.
3916....	.30–.30	.0–.0	.30–.30	Fatty degeneration.	Pernicious anemia.	Acute case.
3742....	.20–.20	.10–.10	.10–.10	Fatty degeneration.	Acute diabetes.	Child 1½ years.
3855....	.35	.15	.20	Slight fatty degeneration.	Diabetes.	

Tables VIII and IX show quite a number of diseases with normal blood lipase. It is of interest that diabetes with definite lipemia shows a normal lipase. Nephritis, acute and chronic, causes no fluctuation in the lipase. Various types of blood disease where we may assume considerable disintegration of blood cells and abnormal marrow activity, show normal blood lipase. All acute infections show a normal lipase except those where the intoxication has caused more or less liver necrosis, focal or diffuse.

TABLE IX.—INFECTIONS.

No.	Acid production.	Alkalinity.	Butyric acidity.	Autopsy liver.	Clinical diagnosis.	Remarks.
3479....	.20-.15	.10-.10	.10-.05	Parenchymatous.	Typhoid.	No liver necroses.
3705....	.20-.22	.10-.10	.10-.12	Slight fatty degeneration.	Lobar pneumonia.	Plasma.
3705....	.35-.30	.10-.10	.25-.20	Slight fatty degeneration.	.... do.....	Serum.
Newton	.40-.35	.10-.10	.30-.25	.....	.... do.....	Toxaemia severe.
3751....	.20-.25	.10-.10	.10-.15	Liver tubercles.	....	Miliary tuberculosis.
3668....	.20-.20	.10-.10	.10-.10	Caseous liver tubercles.	.....	Pulmonary tuberculosis.
3686....	.20-.25	.10-.10	.10-.15	Normal.	.....	Sarcomatosis.
Aro	.20-.20	.15-.15	.05-.05	.....	Normal.	70 years.

dogs. Blood lipase in normal persons or animals is remarkably constant. It may be stated with considerable certainty that liver necrosis, however produced, in a liver previously normal, causes a characteristic rise in blood lipase from 0.2 cc. to 1.2 cc. or even higher—a rise of five to eight times normal. This rise reaches its maximum after twelve to twenty-four hours and may remain at this level for twenty-four hours or longer in fatal cases. The curve of blood lipase then falls slowly to normal on the sixth or eighth day with repair of the injured parenchyma. Small liver injuries with necrosis cause less marked rise in lipase and more prompt return to normal. Very trivial liver injuries even with a little true hyaline necrosis may not give a recognizable rise in lipase. Poisons like chloroform which produce hyaline liver necrosis give a

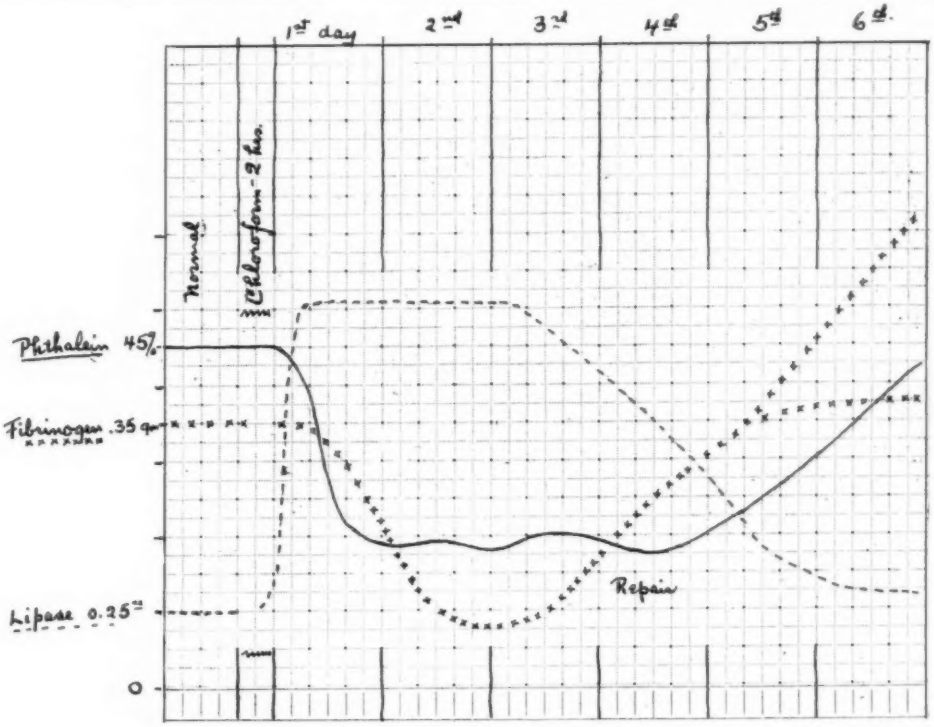

CHART II.

Chart II shows in a diagrammatic way the various changes in phthalein, fibrinogen and lipase which follow a chloroform anæsthesia. Anæsthesia for two hours will cause a central hyalin liver necrosis involving two-fifths to three-fifths of every liver lobule. During the process of repair there is a gradual return to normal and the fibrinogen curve may go far above normal (Whipple and Hurwitz).*

Chart III shows in the same way the reaction following a fatal liver injury by chloroform or phosphorous. Death may supervene on the third to the sixth day. The lipase curve may reach a considerably higher level and the fibrinogen content may fall to zero; the curves represent the average findings.

GENERAL DISCUSSION.

The preceding tabulated observations are quite uniform in the human cases as well as in the experiments performed on higher and more prolonged rise in lipase than other poisons like phosphorous or hydrazine, which cause less true hyaline necrosis and much more fatty degeneration.

Liver necrosis which has been caused by acute intoxication or infection gives a lipase reaction identical with chloroform injury. The liver of fatal chloroform poisoning is very like that of acute yellow atrophy, and it is certain that in the latter instance the blood lipase will be found to be much above normal. The liver necroses associated with eclampsia, leukæmia, pneumonia, septicæmia, etc., all give a constant reaction in blood lipase, depending somewhat upon the extent of the necrosis.

From a study of Table VII it will be noted that this blood lipase reaction to liver injury may not be normal in chronic icterus or advanced cirrhosis. So, too, it will be found that at

times the normal sequence of autolysis of the dead hyaline cells and of inflammatory cellular reaction does not proceed under these conditions. Icterus of long standing may be associated with normal lipase, but at its onset may cause a slight rise above normal. Cholangitis which is associated with parenchyma injury will show a rise of lipase far above normal (Table IV).

Profound changes in blood supply of the liver (Eck fistula, or Eck fistula with hepatic artery ligation) will bring about cell degeneration or even cell death in the latter case and cause a corresponding rise in blood lipase.

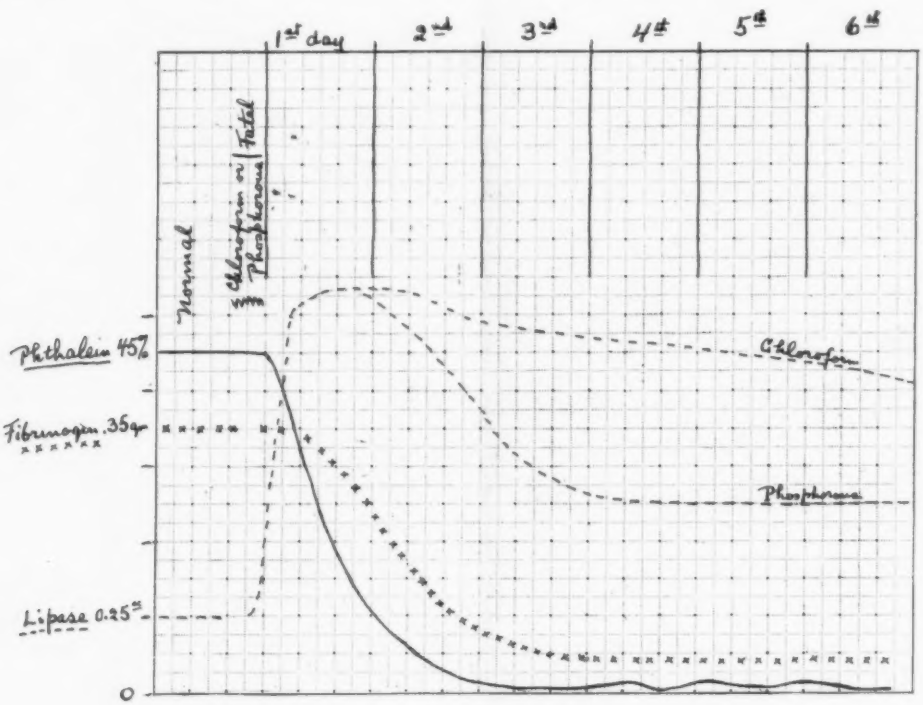

CHART III.

A great variety of acute intoxications not associated with liver injury show no fluctuations in plasma lipase. In this group we may include acute hemorrhagic pancreatitis, acute intestinal obstruction, acute or chronic nephritis with uræmia, leukæmia, pernicious anæmia, diabetes, etc.

Difficulties of diagnosis between true eclampsia and the various toxæmias of pregnancy are common and this test will be of great help, provided the name eclampsia is limited to that type of intoxication associated with portal hyaline liver necroses. One case of true eclampsia (Table V) showed a normal lipase and at autopsy it required the most careful search to reveal a very few small but typical portal hyaline necroses. The very small amount of injured liver tissue was not sufficient to modify the blood lipase. Many cases of suspected eclampsia showed normal lipase and subsequently proved to be chronic nephritis, vomiting of pregnancy with fatty liver, epilepsy, etc.

Finally, it may be stated that a study of the lipase of the blood will show a definite rise above normal in practically all cases of eclampsia, liver injury with necrosis due to poisons, intoxications or infections, acute yellow atrophy, cholangitis and abscess of liver with considerable destruction of liver tissue. Cirrhosis of the liver may show a subnormal lipase; it may react to liver injury by a rise in blood lipase, but this point will require more study.

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